STUDIES ON THE UPTAKE OF PLANT GROWTH SUBSTANCES BY PLANT TISSUES

A

Thesis

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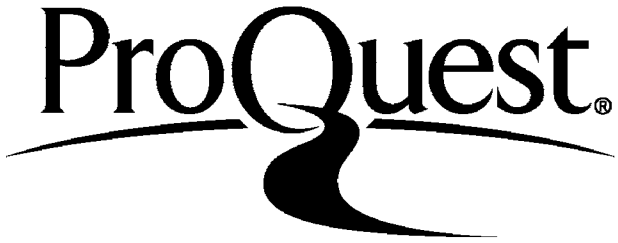


ABSTRACT

The uptake of the auxins indole-3-acetic acid (IAA) and 1-naphthylacetic acid (NAA) was investigated using fluorimetric methods of assay. Uptake by the following plant material was examined - segments of Avena coleoptile, maize mesocotyl and the hypocotyl of Phaseolus radiatus. The growth regulating substances 2,4-dichlorophenoxyacetic acid, triiodobenzoic acid (TIBA), N-1-naphthyl-phthalamic acid, (1-naphthyl-methyl-sulphide) - propionic acid, and 3:5-diiodo- and 3:5-dibromo-4-hydroxybenzonitrile were examined for their effects on the entry and accumulation of IAA and NAA.

Experiments were conducted to distinguish between the initial physical phase of uptake and subsequent metabolic uptake by pretreatment of the tissue with metabolic inhibitors and poisons.

As fluorescence assay can be employed, under the experimental conditions, only for measuring loss of the auxins from the external medium, C^{14}-labelled IAA and I^{131}-labelled TIBA were used to assay actual uptake by the plant material.

As it was suspected that the effects of TIBA on the uptake of IAA were exerted through metabolic action on a sulfhydryl-enzyme system, various SH-enzyme protectors (2,3-dimercaptopropanol, cysteine) were tested in attempts towards tracing the mechanism of TIBA action.

Chromatographic and fluorimetric analysis of variously treated material has been undertaken. Autoradiographic analysis to check the nature and degree of IAA metabolism and the effects of TIBA on it was conducted, and measurements made of respiratory decarboxylation of IAA - C^{14} under the experimental conditions.

The results have been critically discussed with reference to the literature dealing with synergism and antagonism between growth regulators, uptake and transport of auxins, mechanism of auxin action in growth, general aspects of membrane permeability and solute transport, and auxin kinetics.

CONTENTS

INTRODUCTION

INTRODUCTION

The uptake and translocation of auxin by plant tissues have long been regarded as processes which cannot be explained on the basis of diffusion. In the literature dealing with plant growth, one frequently encounters procedures involving the immersion of intact or excised tissues into auxin solutions. However, data concerning the mode of entry of the auxin into the tissue are surprisingly scarce. Since at least a portion of the auxin in the cell must be available for the critical reactions which control the observed responses to external applications of growth regulators, it seems of obvious interest to know the relationships that exist between the growth reaction, internal auxin concentration and the concentration of the external solution. An understanding of the uptake process and the factors affecting it should aid in an eventual comprehension of the mechanism of auxin action.

Early workers regarded the process of auxin absorption by plant tissue as a simple diffusion phenomenon. Albaum, Kaiser and Nestler (1937) investigated the relation of hydrogen ion concentration to the penetration of indole-3-acetic acid (IAA) into Nitella

cells and found that the curve representing the velocity of penetration as a function of pH coincided with the dissociation curve for IAA. They concluded that the auxin entered in the form of the undissociated molecule and the process was in the nature of diffusion. Albaum and Kaiser (1937) estimated the pK value of IAA to be 4.66. Skoog (1938) reported similar effects of pH on the absorption of IAA by tomato roots. The uptake of auxin was proportional to the concentration supplied over a considerable range of concentrations. Working with Cucumis hypocotyls, Sutter (1944) also concluded that uptake of auxin from the medium was the result of diffusion into the tissue. Rice (1948), Fang, et al (1951), Day (1952) and other workers have examined the absorption and translocation of 2,4-dichlorophenoxyacetic acid (2,4-D) by bean plants and other susceptible species. Gallup and Gustafson (1952) examining the absorption and transport of radioactive 2,4-dichloro-5-iodo^{131} - phenoxyacetic acid by various green plants, showed that although species differed in the amounts of the compound that they absorbed over a given period, for all the plants examined the rate of uptake was essentially linear over 96 hours.

The absorption of auxins by plant tissue segments

and slices probably involves the plasma membrane primarily. Increased sorption of 2,4-D through increased H-ion concentration has been reported by a number of workers (Hamner, et al, 1947); Lucas, et al, 1947; Blackman and Robertson-Cunninghame, 1953; Hauser, 1955). An earlier explanation of this acid effect was that at low pH, molecules of weak acids such as 2,4-D and IAA are to a large degree in the undissociated form. In the molecular form, these acids are more likely to partition into fatty phases such as the wax and lipoids of the plasma membrane. However, Blackman and Robertson-Cunninghame (1953) concluded from their experiments with 2,4-D that for weak acids possessing growth-regulating properties, both the molecule and the anion are active. This is in agreement with the findings of Simon and Blackman (1949), Simon and Beevers (1952) and Simon, Roberts and Blackman (1952), who examined the effect of pH on the biological activity of weak acids and bases. They found that although the effect of pH on the degree of dissociation of the acid or base in the external medium is responsible for a large part of the effect of pH on activity, their experimental results indicated that the ions were also effective. Brian and Rideal (1952) found that at a pH above four a compound such as

2, - methyl-4-chlorophenoxyacetic acid is predominantly ionic but still remains highly active. They argue, therefore, that the pH effect of physiological acidities must be largely an effect on the plant rather than on the penetrating compound.

The surface of both cuticle and plasma membrane contains free acid groups from long chain aliphatic acids (cuticle) or amino acid residues (plasma-lemma). The lipoid layer also probably contains acids. These are all weak acids with pK values higher than the penetrating growth substances. It was shown (Brian and Rideal,1952) that the permeability of lipoprotein monolayers to organic acid anions will increase if the negative charge of these layers is lessened by decreasing pH. Increasing H-ion concentrations should, therefore, increase the permeability of protoplasmic membranes to auxin ions.

Reinhold (1954), working with pea stem sections and carrot tissue slices, first demonstrated that the uptake of auxins is a process consisting of two phases. The first step is rapid and physical, and has been attributed to simple adsorption. The second phase is slower, steady, more complex and appears to be metabolic. Reinhold also demonstrated with carrot tissue that the rate of uptake during the initial physical phase was lowered

with increasing pH. This fall did not, however, parallel the fall in the mean concentration of undissociated molecules in the medium. It was suggested that the anion, and not the undissociated molecule, was the form of IAA involved in adsorption and that the decline in rate with increasing pH is due to the ionization of cytoplasmic proteins and protein complexes. The effect of pH on the metabolic process was slight, suggesting that the substrate concentration was always sufficient to saturate the enzyme system.

The results of Johnson and Bonner (1956) indicate that the uptake of 2,4-D by Avena coleoptile sections consists of three separable processes. The first, consummated in 20 - 30 mins., resembled diffusion into the tissue. The second process was also rapid and resembled and exchangeable binding. The third component was a steady, continuing process and the 2,4-D was accumulated against a concentration gradient. The rates of both the initial uptake and the continued uptake were depressed by increasing the pH of the external medium.

From experiments on the uptake of 2,4-D and related compounds by Lemna minor, Blackman, et al (1956, 1959a) demonstrated that the absorption of 2,4-D is characterized by a very rapid rate of entry during the first few minutes followed by a steady rate of accumulation for between

1 and 2 hours. Subsequently the rate falls off to zero and this is followed at higher concentrations by a phase where there is a net loss of the growth regulator to the external solution. Similar results were obtained for the 2 : 6-chloro-substituted compound, save that loss to the solution takes place later. For the 2-chloro-substitution, after the initial phase of uptake there is some loss but this is followed by a period of recovery and a subsequent slow rate of uptake. Phenoxyacetic acid is steadily accumulated. The initial phase of uptake is temperature sensitive and positively and curvilinearly related to the external concentration of 2,4-D. It is also dependent on the pH of the external solution and closely, but not completely, correlated with the external concentration of the undissociated molecule.

Comparable experiments conducted (Blackman and Sargent, 1959 b) to examine the uptake of triiodo-benzoic acid by Lemna minor, indicated a very similar pattern of absorption.

Wedding, et al (1957, 1961) and Swets and Wedding (1964) have examined the effects of a number of factors on the uptake and metabolism of 2,4-D by Chlorella. The uptake of carboxyl-C^{14} - 2,4-D was studied (Wedding and Erickson, 1957) as a function of external pH. Initial uptake consisting primarily of diffusion into the cell and

adsorption within the cell was followed by a slower, 'metabolic' uptake. Increasing pH raised the levels of 2,4-D taken up by the cells relative to the concentration of undissociated molecules in the external buffer medium. An appreciable fraction of the 2,4-D accumulated by Chlorella, a fraction which increases with time, is irreversibly bound in the cell (Wedding and Blackman,1961). The accumulation of this fraction was affected by temperature, light and inhibitors and was therefore considered to be dependent upon a supply of metabolic energy. A single enzymic reaction is postulated as a predominant component of the uptake process for auxins. The presence of IAA and other auxins depressed the uptake of 2,4-D and suggests competition at the site of this reaction.

Increasing concentrations of DNP at first caused a progressive inhibition of the amounts of 2,4-D retained in the cells of Chlorella in a non-diffusible, non-exchangeable form (Swets and Wedding, 1964). Those factors which tend to increase the rate of glycolysis or reduce the activity of the TCA cycle, resulted in an increased uptake of 2,4-D. It was suggested by these workers that acetyl CoA participates in the first reaction undergone by 2,4-D in the cell.

Reinhold (1954) also showed that the metabolic stage of uptake of IAA by pea epicotyls and carrot discs was

highly sensitive to iodoacetic acid, cyanide and arsenite and was considerably depressed by diethyl-dithiocarbamate and 2,4-D.

Johnson and Bonner (1956) found that metabolic uptake of 2,4-D was inhibited by low temperature and by cyanide, DNP and p-chloromercuri-benzoate. The initial diffusion process was little affected by these compounds. Both the exchangable binding and continuing uptake of 2,4-D were inhibited by a high concentration of IAA.

Bennet-Clark and Wheeler (1959) found that the uptake of IAA was inhibited by indoleacetonitrile (IAN) and metabolic poisons, such as cyanide, whereas aspartate promoted uptake. They concluded from their results that the IAA concentration in the sites where it is accumulated exceeds the external concentration of IAA.

Intact and excised corn roots and intact cucumber roots absorbed greater quantities of 2,4-D than of its octyl ester at pH 7. Corn coleoptile sections, however, demonstrated a greater uptake of the octyl ester (Morre and Rogers 1960). These results have been put forward to support the suggestion that roots are more adaptable to the uptake of ionized compounds. In the case of coleoptile sections the greater uptake of the apolar ester over the ionized acid

might be expected.

Thimann and Wardlow (1963) found that high intensity light increased the uptake of IAA by internodal sections of light-grown pea stems and this effect was dependent on the presence of chlorophyll. The effect was thought to be due to a stimulation of locally available photosynthetic energy.

Poole and Thimann (1964) have shown that Avena coleoptile sections floated on solutions of labelled IAA or IAN, rapidly take up radioactivity for the first half hour and then at a nearly linear rate for 4 hours. After 1 to 2 hours, the internal concentration in the tissue exceeded the external, indicating true accumulation. While the uptake of IAA was pH-sensitive, that of IAN was little affected. As much of the activity taken up during the first 30 minutes was lost to unlabelled auxin or water, it was considered to be in solution in the cell walls or other free space of the tissue. The initial physical uptake of IAN was much greater than that of IAA and it was suggested that IAN may be concentrated in some lipophilic part of the tissue. It was concluded that the greater growth activity of the nitrile could be explained by its higher rate of penetration at physiological concentrations.

It has been shown by a number of workers (Reinhold,1954; Johnson and Bonner, 1956; Blackman, et al, 1959a, 1959b;

Swets and Wedding, 1964) that uptake of auxins is temperature sensitive and various Q10 values for IAA, 2,4-D, TIBA and other auxins have been obtained for different plant tissues. Evidence from this work indicates that both the initial phase of entry and subsequent metabolic uptake are affected. Van Overbeek (1956) has pointed out that the primary effect of temperature is exerted on the permeability of the oriented fatty molecules of the plasma membrane.

Whereas anaerobic conditions do not affect the initial uptake of auxins, metabolic uptake is severely depressed. This is additional evidence to indicate that the latter process is dependent on a supply of respiratory energy.

Molecular Structure and Penetration:

The penetration of compounds into plant tissue is much dependent upon their molecular structure. When penetration into and through a fatty phase is involved, a less polar compound has a greater degree of penetration than a more polar compound. Bentley and Housley (1952) suggested that the apparently greater activity of the nitrile, as compared to the more polar acid forms of IAA and 2,4-D, might be a result of greater penetration. Thimann (1953) concluded from his data that in Avena tissue the nitrile was converted enzymatically into the active acid. This conversion was subsequently confirmed and the enxyme, nitrilase, has

been isolated and characterized. (Thimann and Mahadevan, 1958, 1961). Poole and Thimann have recently shown that at physiological concentrations the rate of uptake of IAN is from 2 to 10 times as great as that of IAA, which would explain the greater growth activity of the nitrile observed with Avena. The initial physical uptake of IAN was also larger than that of IAA and suggests that IAN may be concentrated in some lipophilic part of the tissue. For similar reasons, hydrolyzable esters of biologically active weak acids are used in overcoming resistance to penetration (Beevers, et al, 1952; Crafts, 1953).

Until recently it was thought by a majority of workers that the ortho position of the ring of auxin molecules was essential as a point of attachment in the reaction leading to auxin activity (Muir and Hansch, 1951, 1953, 1955). However, Osborne, et al (1954, 1955) have shown that the amide form of 2,6-dichlorophenoxyacetic acid has an auxin activity which the acid form does not have. Furthermore, the addition of an alkyl radical to the alpha carbon of the side chain of 2,6-D also induces auxin activity in the acid. It seems, therefore, that increasing lipoid solubility is all that is necessary to demonstrate auxin activity in this

kind of molecule. Further, it has been suggested that antiauxin activity of compounds may be due to interference with the uptake of auxins rather than solely by competitive inhibition at a growth centre (Ingestad, 1953; Audus, 1954).

Veldstra and Booij (1949) suggested that a proper balance between lipophilic and hydrophilic groupings is an essential requirement for auxin activity. This requirement was re-confirmed by Blackman, Parke and Garton (1955). Facilitated penetration into cells by lipophilic radicals was also demonstrated for antiauxin and defoliant activity of a series of maleimides (Van Overbeek, Blondeau and Horne, 1951).

Partitioning properties of a molecule between a semi-polar lipoid and water are a reasonable guide to penetration into cells (Collander, 1954). In the plasma membrane, however, the lipoid molecules are arranged in micelles which may explain the selective penetration of closely related molecules having the same oil/water partition coefficients.

Permeability of Plant Cells:

According to Danielli (1954) besides osmotic (diffusion) and active uptake, there is yet a third type of absorption which he has termed facilitated diffusion. It resembles simple permeation in so far as both occur under the driving

force of thermal agitation and both reach the same equilibrium. On the other hand, facilitated diffusion is restricted by both structural and steric factors and is thus pronouncedly specific in nature. Competition for entry takes place between structurally related compounds, and certain specific poisons can bring about cessation of penetration (Jennings, 1963). The state of penetration is also many times greater than can be expected if the molecule were passing through a homogeneous lipoid membrane.

It is now assumed in some quarters that the plasma-lemma is much more permeable, at least to ions, than the tonoplast. It is also assumed that the apparent free space (Epstein, 1956; Briggs and Robertson, 1957) consists not only of cell walls and intercellular spaces but also includes part of the protoplasm (mesoplasm). It would follow, therefore, that the plasma-lemma must be readily permeable to numerous solutes which only very slowly penetrate the tonoplast. However, the observations of Bennett and Rideal (1954) suggest that the resistance of the outer plasma membrane is by no means negligible as compared with the resistance of the inner one.

Collander (1957) stresses that the negative correlation found between molecular weight and permeation power

(Collander 1954) does not prove that the molecular size as such is a factor directly concerned with the permeation process. Similarly, the empirically found correlation between permeation power and lipoid solubility does not constitute conclusive proof that permeants enter or leave the protoplast by truly dissolving in the plasma membrane lipoids. It does indicate, however, that the permeation process consists of events which are at least closely connected with solubility, and probably primarily controlled by intermolecular forces identical with those which control the partition of a solute between two mutually immiscible solvents.

The view that permeation power is primarily related to the undissociated acid and base molecule is supported by the investigations of Bennett (1955) on the toxicity of growth substances to Nitella, and the work of Erickson, et al (1955) on the influence of acetic acid and 2,4-D on Chlorella. Bennett remarks, however, that his results are understandable only if we assume that the pH at the surface of the plasma membrane is about 1.1 units lower than the pH value in the bulk solution. This observation is consonant with Danielli's statement that the difference between bulk and and surface concentrations of hydrogen ions may be of the

order of several pH units and hence may be of great importance in biological processes.

Transport:

The transport system that has been shown to exist in plant tissue for the translocation of some auxins must necessarily be closely linked to the uptake process. Only a few auxins are known to exhibit strict polarity in the transport system. These include IAA (Jacobs, 1950; Niedergang, 1954; Leopold and Guernsey, 1953; Zwar and Rijven, 1956; Hay, 1956; Niedergang-Kamien and Skoog, 1956; Niedergang-Kamien and Leopold, 1957, 1959; Leopold and Lam, 1962; Leopold, 1963; Hertel and Leopold, 1963; McCready and Jacobs, 1963a, 1963b), Naphthaleneacetic acid (NAA) (Leopold and Lam, 1961; Leopold, 1963), 2,4-D (Hay and Thimann, 1956; McCready, 1963; McCready and Jacobs, 1963a, 1963b), indole-3-butyric acid (Leopold and Lam 1961). The extent of transport polarity varies over a very wide range, from the essentially completely polar coleoptiles and young stems to the weakly polar petioles and roots. Variations in the relative polarity of auxin transport between tissues and between plant parts at various stages of development or following tropistic stimulation may involve differences in the directional feature of active movement. Many synthetic auxins are

apparently incapable of being transported in the IAA system, including many phenoxyacetic acids, benzoic acids, phthalamic acids, and others. These synthetics are generally effective inhibitors of the transport of IAA or other auxins.

That the movement of auxin is accomplished by an active transport system is indicated by the following features which satisfy the criteria for an active transport mechanism according to Danielli (1952). That auxin transport is faster than diffusion is perfectly evident from the velocity determinations for IAA and 2,4-D mentioned by a number of workers. That it is selective for some substances is likewise evident from the restriction of polar transport to IAA, NAA, 2,4-D, and a few other auxins. That polar transport of auxin can move auxin against a concentration gradient has not been established unequivocally, but there is much evidence to suggest this. (Goldsmith, 1959). Lastly, numerous workers have reported the sensitivity of the auxin transport mechanism to metabolic inhibitors and poisons.

Hertel and Leopold (1962, 1963) have concluded that the auxin pump is secretive in nature, moving the molecules preferentially outwards from the cells. McCready and Jacobs (1963 b) showed that IAA enters into petiole sections

of Phaseolus vulgaris at equal rates, whether supplied at the apex or base of the section, whereas the exit of the auxin is essentially restricted to the basipetal direction. Leopold (1963) considers this to be additional evidence consistent with the concept that the polarity of auxin transport is a consequence of a secretive function and not of an uptake function.

Studies of transport processes involved in the movement of sugars, amino acids, etc., in other biological systems have shown these processes to have the characteristics of active transport systems (Rothstein, 1954; LeFavre, 1954; Birt and Hird, 1956; Bieleski, 1960 a, 1960 b, Johnstone, 1964) and the experiments have permitted a limited amount of analysis of the entry and exit rates of these substrates. In each of these systems it has been deduced that there is an attachment of the transported substrate to some entity in the membrane, and kinetic studies of the rates of entry and exit of substrates have permitted some development of evidence for enzymatic actions in the attachment and disengagement of the substrate from the transport site.

It has also been suggested that auxin transport involves a stereo-specific site of attachment on the basis

of the inhibitory effects of auxin anologues (Niedergang-Ramien and Leopold, 1959; Keitt and Skoog, 1959).

It has now been realized that only a small portion of the auxin taken up by tissues is effectively transported, the rest remaining in a static or non-transported pool (Goldsmith and Thimann, 1962). The static pool varies in size with different transporting tissues (Gillespie and Thimann, 1963). Evidence for the existence of this static pool is also displayed by the results of Scott and Briggs (1962), Leopold and Lam (1962) and Hertel and Leopold (1963).

Auxin Metabolism:

The literature dealing with the mechanism of action of auxin and the metabolism of endogenous and exogenously supplied growth substances is extremely abundant, and numerous reviews have appeared on the subject (Audus, 1949, 1961; Bonner and Bandurski, 1952; Gordon, 1954; Muir and Hansch, 1955; Aberg, 1957; Bentley, 1958, 1961; Galston, et al, 1960, 1961; Fawcett, 1961; Hilton, et al, 1963; Fredga and Aberg, 1965). It will, therefore, be only very briefly touched upon here in so far as it is a factor to be taken into consideration in studies on the uptake of auxins.

The data for the metabolism of auxins have been derived almost entirely from feeding experiments. The destruction of IAA may take place in a variety of ways. Photo-oxidation may take place in the light with the formation of the inactive aldehyde (Galston, 1949). IAA may also be degraded in the dark by a number of different enzymes including oxidases, peroxidases and catalase with the formation of a number of oxidation products (Galston, Bonner and Baker, 1950, 1953; Houff, et al, 1954; Manning and Galston, 1955; Ray and Thimann, 1955; Waygood and Maclachlan, 1956; Galston and Hillman, 1961). Galston and Dalberg (1954) postulated the formation of an adaptive IAA-oxidase induced by auxin feeding.

Auxins are also thought to form complexes with normal metabolites in the cell. Thus Siegel and Galston (1953) reported the formation of an IAA-protein complex by pea roots which had been incubated in high concentrations of IAA. Freed, et al (1961) proposed that the predominant mechanism of action for auxins is adsorption on a protein surface, with modification of the protein structure in such a way as to change its enzymatic activity. That the locale of auxin action may be in the non-particulate phase of the cytoplasm is further evidenced by the findings of

Galston and Kaur (1961 a) that 2,4-D and IAA decreased heat coagubility of cytoplasmic proteins of pea stems and roots without altering total protein content. Results of subsequent work (Galston and Kaur, 1962) showed that the altered coagubility persisted after dialysis and indicated that the altered substance was a macromolecule.

In a series of publications Andreae and co-workers (Andreae and Good, 1955, 1957; Andreae and van Ysselstein, 1956, 1960a, 1960b; Good, Andreae and van Ysselstein,1956; Andreae, Robinson and van Ysselstein, 1961) reported that IAA was conjugated with aspartic acid and ammonia during its metabolism in the plant. Zenk (1961, 1964) subsequently demonstrated that the indole-3-acetamide found by Andreae was an experimental artefact. Zenk also reported a new IAA conjugate in plants, 1-(indole-3-acetyl)-B - D- Glucose. Klämbt (1961 b) established the presence of aspartic acid and glucose-ester derivatives of 2,4-D in wheat coleoptile cylinders and considered the latter a detoxication product. Benzoic acids also formed conjugation products with glucose in wheat coleoptiles, metabolic products being D-glucose-1-benzoate and D-glucose-6-benzoate. (Klämbt 1961a). A number of other workers (Holley, Boyle and Hand, 1950; Holley, 1952; Stutz, 1958; Bennet-Clark and Wheeler, 1959; Fang, Thiesen and Butts, 1959; Bach, 1961) have reported

metabolic complexes and detoxication products formed in plant tissue treated with IAA, 2,4-D and other growth substances. Leopold and Plummer (1961) reported the formation of auxin-phenol complexes.

Hilton, et al (1963) point out that the metabolic fate of phenoxyacetic acids includes (a) Physical or chemical conjugation with cellular constituents (Galston and Kaur, 1959; Brian, 1960; Khubatiya, 1960; Klämbt, 1961 b), (b) degradation of the aliphatic side chain of the molecule (Wain, 1958; Fawcett, et al, 1959; Canny and Marcus, 1960; Luckwill and Lloyd-Jones, 1960-, (c) ring hydroxylation (Bach, 1961).

It is now established that the homologous series of aryl- or aryloxy-acids, with long straight side chains, can be degraded by β - oxidation, either to the corresponding phenol or to the acetic acid depending on the number of carbon atoms in the side chain. This ability varies from plant to plant and is a function of the structure of the auxin ring (Fawcett, Ingram and Wain, 1952, 1954; Wain and Wightman, 1954; Wain, 1955 a , 1955 b).

Numerous attempts have been made to characterize the primary site of auxin action. Hansch and Muir (1961) proposed that growth regulators act via two-point attachment

with a plant substrate. In a study of 19 monosubstituted phenoxyacetic acids, they concluded that one point in the substrate is probably an amino group. The primary mode of auxin action has been visualized by Van Overbeek (1961) to involve physico-chemical bonding; to include sorption on the lipoprotein membrane of the cytoskeleton rather than on a key enzyme system; and to occur on oil soluble particles rather than in aqueous systems.

Heath and Clark (1956, 1959, 1960) suggested that activity of certain growth regulators may be in part due to their metal-chelating properties. The work of Perrin (1961) indicates that for IAA and acetic acid only the carboxyl group plays any part in complex formation. Studies in stability constants of copper complexes of various phenoxyacetic acids (Armarego, 1959) and ultra-violet absorption spectra at various pH levels (Fawcett, 1959) fail to support the hypothesis that growth regulators act by chelation. A comprehensive discussion pertaining to the problems involved in chelation has been presented by Thimann and Takahashi (1961).

The possibility of the activity of various herbicides being limited by their adsorption to sites not concerned in the physiological process was investigated (Brian, 1960)

by measuring adsorption to oat monolayers. A considerably greater adsorption was obtained with 2, 4, 5 - and 3, 4 - chloro benzoics than with the more active 2, 3, 6 - and 2, 6 - derivatives.

From the brief review given above it will be evident that the uptake of plant growth substances is a function of various processes going on within the plant and is dependent on a number of factors including the structural and steric relations between growth substances and receptor entities in the tissue, permeability, transport, etc. A number of systems also exist for the metabolism, degradation and detoxication of applied growth substances and these need to be carefully considered before any arbitrary assumptions are made relating internal auxin concentrations within the tissue to concentrations of externally applied auxins.

In the present investigation the uptake interactions of growth substances in plant tissue have been studied employing methods of spectrophotofluorometry and radioactive tracers.

The uptake of the auxins IAA and NAA was examined using techniques of fluorescence assay. As these methods could be employed only for measuring loss of the auxins from

the external solution, C^{14} - labelled IAA and I^{131} - labelled TIBA were used to assay uptake by the plant material. The following plant material was selected for study:- Segments of Avena coleoptile, maize mesocotyl, and hypocotyl of Phaseolus radiatus. The growth-regulating substances 2,4-D, TIBA, N-1-naphthyl-phthalamic acid (NPA), (1-naphthyl-methyl-sulphide) propionic acid (NMSP), Ioxynil and Bromoxynil were examined for their effects on the entry and accumulation of IAA and NAA.

Experiments were conducted to distinguish between the initial physical uptake and subsequent metabolic phase of uptake by treatment of the tissue with various metabolic inhibitors and poisons. Attempts were particularly directed towards tracing the mechanism of uptake of auxins and the interactions of growth substances during the uptake process in the tissues investigated.

The experimental techniques have been fully described under the chapter entitled Material and Methods. The results have presented in graphical as well as tabular form wherever possible, and have been critically discussed with reference to the pertinent literature in the field.

MATERIAL & METHODS

MATERIAL AND METHODS

The plant material selected for study in the present investigation consisted of segments from the coleoptile of *Avena sativa* L., the mesocotyl of *Zea Mays* L. and the hypocotyl of *Phaseolus radiatus* L. The *Avena* coleoptile was selected because of its wide use in growth experiments and the latter two for their anatomical diversity. All three were found to be suitable material for studies on uptake of growth substances from solution. The methods used for growing and harvesting the plants are described below:

Avena sativa L. var. Victory II.

The seeds were washed and soaked in tap water for three hours. They were then sown in 1" of thoroughly washed, drained sand in covered glass half bricks and allowed to germinate in the dark at 25°- 26° C. After 72 hours, the seedlings were exposed to 3 hours of red light in order to suppress elongation of the mesocotyl. At 92 hours, plants of approximately the same height were harvested and a segment 10-mm in length was cut 3-mm below the tip of each coleoptile. In selecting coleoptiles, care was also taken to see that the primary leaf filled up the cavity within the coleoptile cylinder in order to minimise variations due to this factor.

Johnson and Bonner (1956), using the same tissue in their work, reported that the leaves do not take up 2,4-D appreciably and therefore did not remove them from their sections. The presence of the leaf prevents the auxin from being trapped in the central hole. Pooled and randomized segments were distributed in lots of generally 100 segments per treatment. Harvesting, cutting and all subsequent manipulations were carried out under dim red light.

Zea mays L. var. Giant White Horse Tooth.

Maize seeds were soaked for 48 hours in an aerating washer (Audus, 1956) at a temperature maintained between 22°- 26° C. Sprouting seeds were carefully chosen for size and prominence of the emerging coleoptile and sown in sand in covered glass half bricks. They were then allowed to germinate in the dark at 25°C for a further 90 hours. On the sixth day, plants of uniform height were harvested and two 10-mm segments were cut from the mesocotyl (first internode) of each plant. As in the case of Avena, all manipulations were performed under dim red light. Lots of 50 segments were generally used per treatment.

Phaseolus radiatus L.

Seeds were washed and soaked in running tap water for 24 hours at 22°- 26° C. They were then sown in moist sand

or vermiculite and allowed to grow in the dark at 25°- 26° C. At 114 hours (on the 5th day) plants of uniform height were harvested and 10-mm segments were cut from the hypocotyl approximately 10-mm below the cotyledons. Generally lots of 50 segments were used per treatment.

Preparation of Experimental Solutions:

Aqueous stock solutions of all the compounds used were prepared at concentrations of 5×10^{-4} M or 10^{-3} M. Those compounds that were less readily soluble in water were first dissolved in a minimum volume of methanol. TIBA was converted into its ammonium salt, excess ammonia was neutralized with I M citric acid and the aqueous solution made up to volume. Ioxynil (3:5 - diiodo - 4 - hydroxybenzonitrile) was used in the form of its sodium salt and Bromoxynil (3:5 - dibromo - 4 - hydroxybenzonitrile) as the potassium salt. All experimental solutions were made up to the desired concentrations in 0.01 M phosphate-citrate buffer at pH 5.0.

EXPERIMENTAL METHODS: (a) FLUORESCENCE ASSAY:

After cutting, the segments were equilibrated for one hour in the phosphate citrate buffer. Lots of 50 to 100 segments were randomly removed from the buffer, superficially

dried on filter paper, and transferred to the experimental solutions comprising individual treatments. Each batch of segments was floated on 10 ml of medium in 3" Petri dishes. During the experimental period, the Petri dishes were agitated on a mechanical rocker and maintained in the dark at 25°C ± 1°C. At periodic intervals specified in the data, 0.5 ml samples were pipetted out from each Petri dish, transferred to 1" x 1" vials and diluted for fluorescence assay with 1.5 ml of buffer. Concentrations of the growth substances used in the present investigation were maintained between 5×10^{-6}M and 10^{-4}M so as to minimise side-effects resulting from supra-optimal or toxic levels of concentrations.

Operational Techniques:

Quantitative assay of fluorescence was carried out employing an Aminco-Bowman spectrophotofluorometer. The construction and operation of this instrument has been fully described by Udenfriend (1962) and will only be briefly outlined here. In operation, light from a Hanover xenon arc with a D.C. ballast is dispersed by the exciting monochromator into radiation incident on the sample. Fluorescent light from the sample is dispersed by a similar

monochromator into a monochromatic beam which shines on a photo-multiplier detector. A weak electric signal is fed from the detector to a photometer where it is amplified. The photometer output is coupled to the vertical axis of a cathode-ray oscillograph. For quantitative analysis, the signal can be indicated directly on the meter. The two gratings are oscillated by motor-driven cams to which are coupled graduated discs for visual observation and manual adjustment of wave-length. Potentiometers coupled to the gratings supply the wavelength information to the X-axis of the oscillograph in the form of a D.C. signal.

It was found that the wavelength scale for the fluorescence monochromator was not correctly aligned with the grating. This was carefully calibrated using a solution of quinine sulphate in decinormal sulphuric acid at a concentration of 1 ug/ml. Scattered light, reflected from a piece of ground glass in the cell, revealed that the misalignment error was uniform over the wavelength scale. A constant correction factor was applied to all the observed fluorescence wavelengths.

In the present work with Avena and Zea tissue, interference due to the presence of factors other than the growth substances under study was minimal. Therefore, relatively

wide slits were used in the optical system, for both incident and emergent beams, to provide maximum sensitivity for detecting the small changes in the concentration of the experimental solutions. Narrowing the width of the slits increases the precision of wavelength determination but lowers the sensitivity of the instrument. After preliminary scanning of the test solutions for interference, the wavelengths of the incident and fluorescent beams were set to the known maxima and the wide slits were used in all further measurements.

The inherent instability of the xenon-arc is reflected in a tendency to wander. This means that small variations occur in the total light incident on the test solutions. The use of relatively wide slits and frequent comparisons between samples and standards reduces this variation to less than 5%. An IP 28 photomultiplier tube was used for all measurements. Udenfriend (1962) and Burnett and Audus (1964) have discussed various factors which can affect the fluorescence of irradiated solutions. These discussions, however, are more relevant to the analysis of plant extracts and will be omitted here.

Emission and Fluorescence Maxima:

Teale and Weber (1956) report that IAA exhibits the

characteristic fluorescence of the indole group; excitation maximum at 287 mμ and fluorescence maximum at 348 mμ (corrected). Hornstein (1958) records an excitation maximum of 285 mμ and a fluorescence maximum of 345 mμ for IAA in aqueous solution at pH 7, and values of 282 mμ and 327 mμ for NAA in aqueous solution at pH 11. Sprince, et al (1957) list many indole derivatives which have fluorescent characteristics similar to IAA. For all the indoles the fluorescence yield varies with pH and is lowest in strong acid and base (White, 1959). Udenfriend (1962), mentioning the results of Teale and Weber, has stated that the excitation maximum of IAA is 278 mμ. In quoting Udenfriend, this error has been repeated by Khalifa, et al (1963). Lewis, Khalifa and Coggins (1965) obtained values of 290 mμ (excitation wavelength) and 360 mμ (fluorescence wavelength) for IAA, but do not mention whether these are corrected values. For α-naphthaleneacetic acid, Khalifa, et al (1963) report values of 310 mμ (excitation maximum) and 340 mμ (fluorescence maximum). Burnett and Audus (1964) obtained values of 285 mμ and 365 mμ, for the two maxima respectively, for IAA in phosphate-citrate buffer at pH 5.0, using the Aminco-Bowman spectrophotofluorometer. These results were checked in the present work and identical values were

obtained. For 1-naphthylacetic acid the excitation maximum was found to be 290 mμ and the fluorescence maximum 336 mμ (corrected).

During fluorimetric measurements, care was taken to ensure that photo-decomposition and temperature fluxes were kept at a minimal level. All chemicals used were of Analytical Reagent grade. A standard quinine sulphate blank was measured with every set of test samples.

Standard Calibration Curves:

Standard calibration curves were prepared for IAA and NAA to estimate the range of concentrations over which Beckman's Law was followed. The lower limit of sensitivity set by solution scatter is about 2×10^{-8}M for IAA and concentrations up to ca. 3×10^{-5}M can be measured before the curve starts to flatten as a result of self-quenching. This degree of sensitivity is equal to most biological assays but the accuracy and specificity of the technique is very much greater. Fig. A shows a calibration curve prepared for standard solutions of IAA ranging from 7.5×10^{-7}M to 10^{-5}M. Fig. B presents a similar curve obtained with concentrations of NAA within the same range. In the case of this compound the curve begins to flatten at a concentration of approximately 1.25×10^{-5}M.

FIG. A

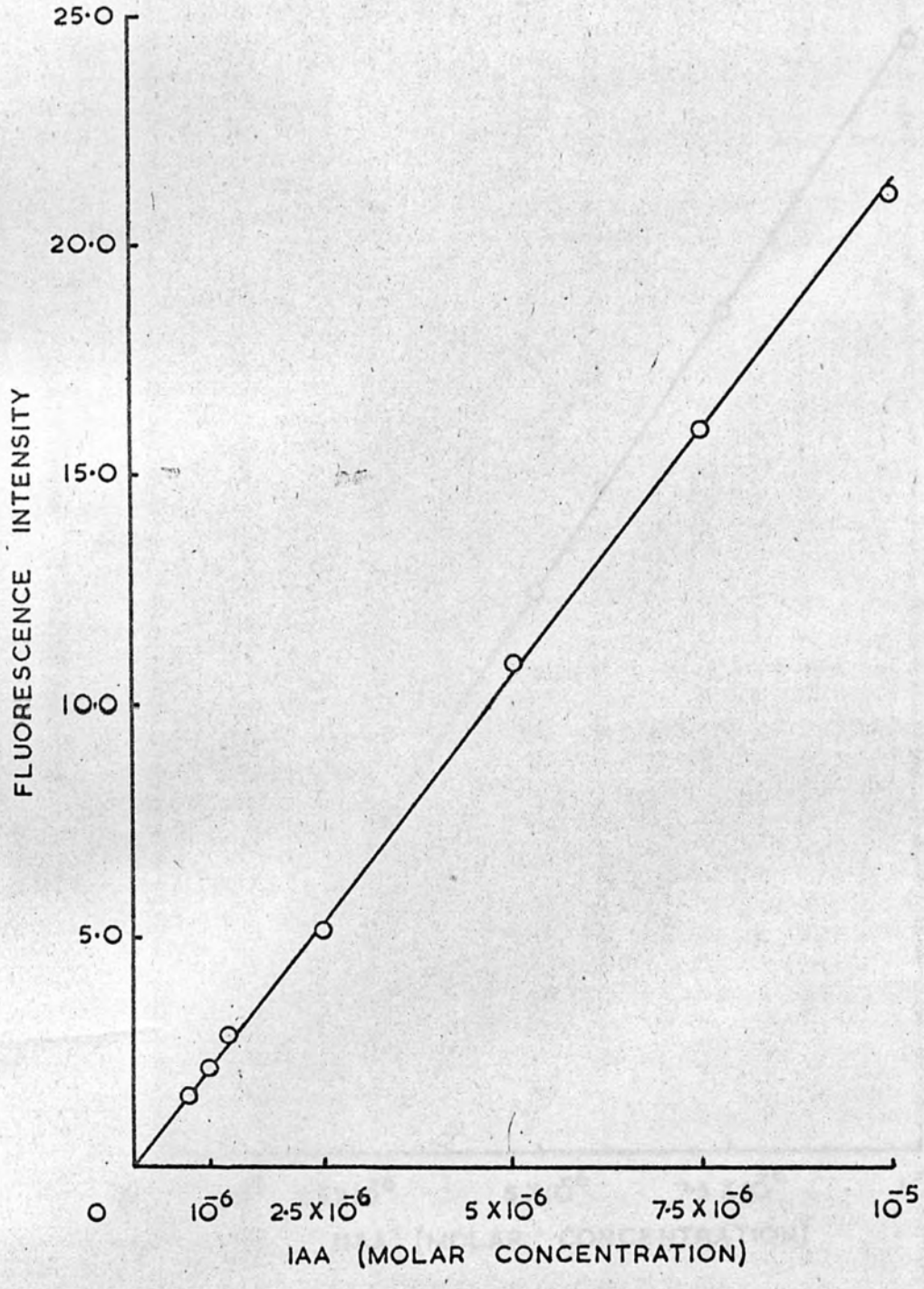
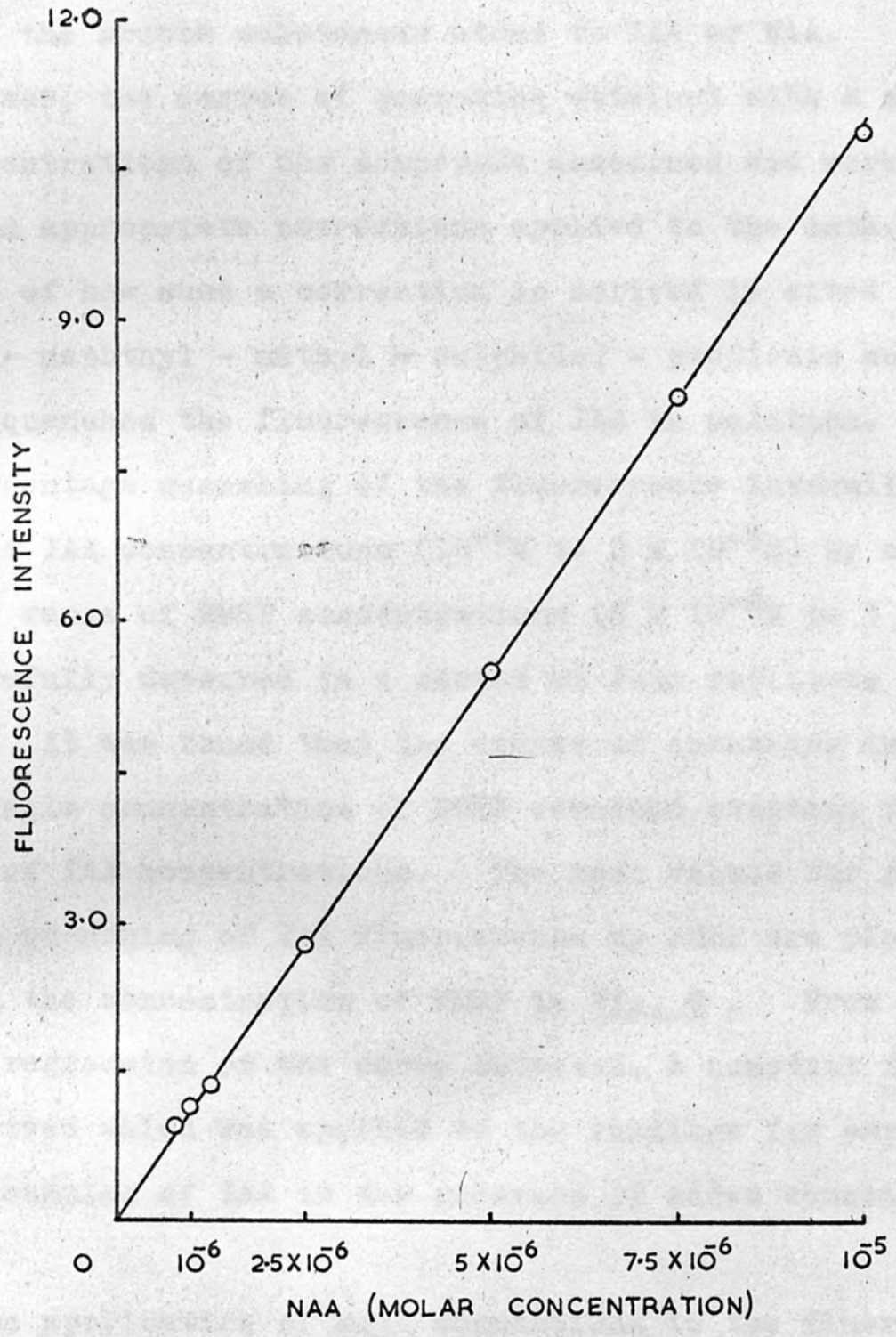

25·0
20·0
15·0
10·0
5·0
FLUORESCENCE INTENSITY
0
10^{-6}
$2{\cdot}5 \times 10^{-6}$
5×10^{-6}
$7{\cdot}5 \times 10^{-6}$
10^{-5}
IAA (MOLAR CONCENTRATION)

FIG. B

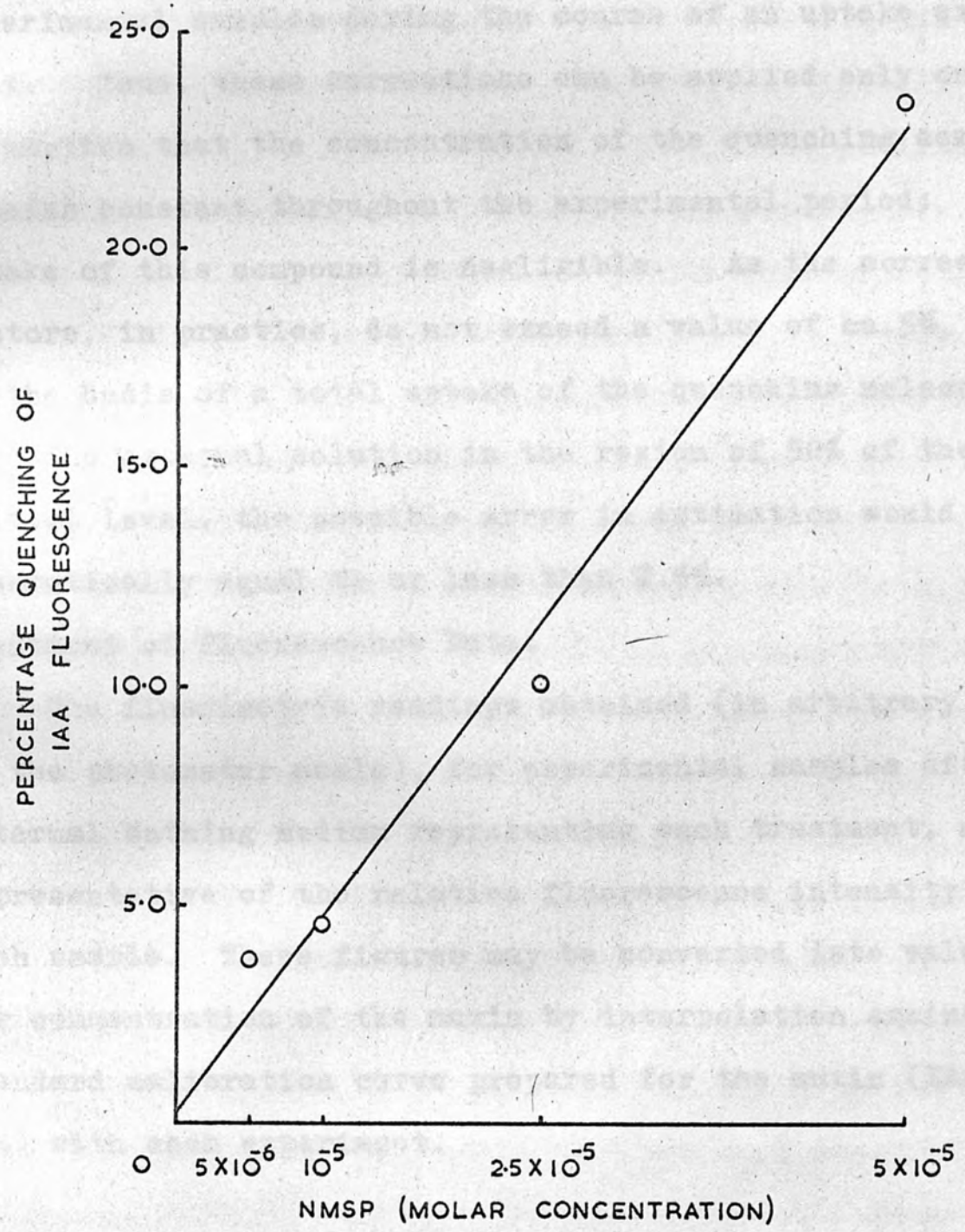

12·0
9·0
6·0
3·0
FLUORESCENCE INTENSITY
0
10^{-6}
$2{\cdot}5 \times 10^{-6}$
5×10^{-6}
$7{\cdot}5 \times 10^{-6}$
10^{-5}
NAA (MOLAR CONCENTRATION)

Corrections for Quenching of Fluorescence:

Quenching of fluorescence intensity was evident with some of the growth substances added to IAA or NAA. In such cases, the degree of quenching obtained with a series of concentrations of the compounds concerned was worked out, and appropriate corrections applied to the data. An example of how such a correction is derived is cited below: α - (1 - naphthyl - methyl - sulphide) - propionic acid (NMSP) quenches the fluorescence of IAA in solution. The percentage quenching of the fluorescence intensity of a range of IAA concentrations (10^{-6}M to 2 x 10^{-5}M) by a similar range of NMSP concentrations (5 x 10^{-6}M to 5 x 10^{-5}M) was carefully measured in a series of four replicate experiments. It was found that the degree of quenching induced by a single concentration of NMSP remained constant for the series of IAA concentrations. The mean values for percentage quenching of IAA fluorescence by NMSP are plotted against the concentration of NMSP in Fig. C . From the linear regression of the curve obtained, a constant factor was derived which was applied to the readings for experimental samples of IAA in the presence of added concentrations of NMSP.

The application of such corrections to the fluorimetric

FIG. C


25·0
20·0
15·0
10·0
5·0
PERCENTAGE QUENCHING OF IAA FLUORESCENCE
0
5X10⁻⁶
10⁻⁵
2·5X10⁻⁵
5X10⁻⁵
NMSP (MOLAR CONCENTRATION)

readings considerably erased the variations obtained due to quenching, but may contain a small error inherent in their application to the fluorescence measurements taken of experimental samples during the course of an uptake experiment. Thus, these corrections can be applied only on the assumption that the concentration of the quenching compound remains constant throughout the experimental period; i.e. uptake of this compound is negligible. As the correction factors, in practice, do not exceed a value of ca.5%, even on the basis of a total uptake of the quenching molecules from the external solution in the region of 50% of the initial level, the possible error in estimation would be theoretically equal to or less than 2.5%.

Treatment of Fluorescence Data:

The fluorimetric readings obtained (in arbitrary units of the photometer scale), for experimental samples of the external bathing medium representing each treatment, are representative of the relative fluorescence intensity of each sample. These figures may be converted into values for concentration of the auxin by interpolation against a standard calibration curve prepared for the auxin (IAA or NAA) with each experiment.

In the present investigation, the external medium was sampled both just prior to adding the tissue segments (sample termed - "Initial - a") and immediately after immersing the tissue ("Initial - b"). It was found that the fluorimetric readings obtained for "initial-b" often tended to be slightly higher than those obtained for "initial - a" for any one treatment, the figures for the latter generally being very close to the expected values on the basis of the standard calibration curves prepared with the experiments. The reasons for this discrepancy are rather obscure. It might possibly be attributed in part to a sudden small influx of water from the external medium into the tissue segments following immediately on their immersion. This would result in a proportionate increase in the auxin concentration, reflected in a rise in the intensity of fluorescence recorded. In the case of experiments with the Avena coleoptile especially, the magnitude of the difference between "Initial - a" and "Initial - b" amounted to as much as 8% to 10%. If uptake was to account for all of this difference, it would involve the absorption, from 10 ml of solution, of up to 1.0 ml of water by a volume of tissue that was well within 2.0 c.c. This, of course, is an obvious impossibility. Some other factor, therefore, must be largely responsible for this effect.

Under the above mentioned conditions a calibration curve prepared with a separate set of standard solutions cannot be employed for estimations of auxin concentrations. As a consequence it was assumed that "Initial - b" represents the fluorescence intensity of an auxin solution of known concentration at the start of an uptake experiment. The concentrations of subsequent samples, therefore, were calculated directly from this reading. This method has been used throughout the present work as it represents a more accurate estimation of loss of auxin due to uptake from individual experimental solutions. From the values for concentration obtained in this way, uptake in absolute amounts may be calculated, after allowing for the periodic sampling of the media. In the results, these uptake values have been expressed in micrograms of the auxin per 100 Avena coleoptile segments, 50 mesocotyl segments of Zea or 50 hypocotyl segments of Phaseolus respectively. Where the number of segments used per treatment has been altered in some experiments, this has been specifically indicated.

EXPERIMENTAL METHODS: (b) ASSAY OF RADIOACTIVITY:

The estimation of uptake by fluorescence assay of the external Solution has certain critical limitations. One

has to work on the assumption that uptake of water from the external solution by the plant tissue is so negligible as not to effect any significant changes in the concentration of the growth substance in the external medium during the experimental period. Secondly, one has to assume that the content of endogenous diffusible or exchangeable auxin is too small to interfere with the assay. Thirdly, the presence of other endogenous factors capable of diffusing out of the tissue can seriously affect the assay. Tests conducted to estimate the extent of this interference, by incubating the segments in distilled water or buffer solutions, are not sufficient to suggest that the presence of physiologically high concentrations of growth substances in the external medium does not induce exchange with such interfering factors. A more reliable, but still inconclusive test, is to incubate the plant material in a medium containing equimolar concentrations of a non-fluorescing compound of similar molecular structure, physical properties and comparable auxin activity. This, however, is not often possible. The extent of these limitations, of course, varies extensively from tissue to tissue. This is illustrated in the results of the present work where although the fluorescence assay methods were applicable

to studies on the uptake of IAA by Avena coleoptile and Zea mesocotyl tissue, in those experiments employing segments of the hypocotyl of Phaseolus radiatus, the presence of an unknown highly fluorescent endogenous factor diffusing out of the tissue, completely precluded any assay of uptake by fluorimetry.

Nevertheless, it must be pointed out that fluorimetry is an extremely sensitive method of assay, and used in conjunction with a technique for measuring absolute uptake by the tissue, it can provide a large amount of valuable information. In this role it can aid in determining the nature of diffusible endogenous factors, the rate of exchange, the purity of solutions and, perhaps most important of all, it provides a sensitive technique for estimating the concentration of fluorescing compounds in solution.

In view of the above, it was decided to examine actual uptake of auxins by the tissue using radioactive compounds. The uptake of IAA-C^{14}, labelled in the carboxyl group, and of TIBA tagged with I^{131} was investigated using isotopic tracer techniques. A Panax, solid-phosphor, scintillation counter was used for a series of experiments conducted with Avena coleoptile segments. Although the efficiency of this instrument is fairly high, counting individual planchets

was laborious and time-consuming. Consequently, for all the experiments carried out with Zea and Phaseolus tissue, an IDL automatic Betamat with an end-window geiger detector was employed. This instrument is equipped with an automatic sample changer on which over 50 planchets may be loaded. The samples can also be continuously re-counted by the use of an automatic multi-cycling device. Methods for the assay of radioactivity and interpretation of activity measurements have been discussed by the authors of a number of texts among which Kamen (1951), Faires and Parks (1958) and Francis, Mulligan and Wormal (1959) were extensively referred to.

Preparation of Radioactive Stock Solutions:

Ampoules of radioactive indole-3-acetonitrile (IAN - C^{14}), labelled in the nitrile group, were obtained from the Radiochemical Centre, Amersham. The specific activity of the IAN was 0.1 mc per 1.34 mg. After dilution with "cold" IAN, this was converted to IAA-C^{14} by alkaline hydrolysis. The IAA thus obtained was extracted and made up to the desired stock concentration. The method of preparation is described in detail below:-

The radioactive IAN (1.34 mg) was diluted with 6.5 mg of cold IAN and hydrolyzed with normal NaOH for 45 minutes over a water bath. The hydrolyzate was cooled and

acidified to pH 3.0 with 10% w/v of H_3PO_4. It was then extracted with three times its volume of ether. The aqueous layer was drawn off and re-washed with ether. The ethereal fractions were combined and evaporated down to dryness under vacuum. The residue of IAA - C^{14} was taken up in distilled water and an aqueous stock solution was prepared with a final concentration of approximately 5×10^{-4}M and specific activity of 1.0 mc/0.5 mM. At every stage of the extraction procedure, the various discarded fractions were examined for residual activity, and re-extracted if necessary. Recovery was almost complete, loss during the entire procedure being estimated to be less than 2%.

The IAA - C^{14} thus prepared was analysed for chemical and radiochemical purity by standard techniques of chromatography and autoradiography. Ascending chromatography was used and the solvents employed were (a) iso-BuOH: MeOH: H_2O: NH_4OH (80 : 5 : 15 : 1) and (b) iso-PrOH: NH_4OH: H_2O (10 : 1 : 1). Autoradiograms indicated the presence of a single radioactive spot whose Rf corresponded with that obtained for synthetic marker IAA. Spraying the chromatograms with the Salkowski or Ehrlich reagents, again revealed the presence of a single reactive region with the same colour and position as synthetic IAA. Careful fluorimetric

analysis, however, demonstrated traces of a fluorescing impurity. Although the fluorimetric readings were of approximately the expected order of magnitude, the fluctuating interference prevented any precise determination of IAA - C^{14} concentration. Although great care was taken during the procedure of hydrolysis and extraction, it is possible that traces of an impurity present in the original sample of radioactive IAN were carried over to the IAA stock solution finally prepared.

TIBA - 2 - I^{131} was also obtained from the Radiochemical Centre, Amersham. This was diluted with non-radioactive TIBA, converted to the ammonium salt with a minimum volume of NH_4OH, the excess ammonia was neutralized with I M citric acid, and an aqueous stock solution prepared to a final concentration of 5×10^{-4} M and a specific activity of 1.0 mc/0.125 mM.

Experimental Procedure for Uptake.

Avena coleoptile:

The general procedure for measuring uptake of IAA-C^{14} by Avena coleoptile segments was similar to that described earlier for the estimation of uptake by fluorescence assay. Lots of 50 segments were immersed in 5.0 ml of the radioactive test solution in glass Petri dishes. At each sampling

interval during the course of an experiment, one lot of segments was removed from solution and superficially blotted on filter paper. The segments were then transferred to muslin gauze stretched over a beaker, briefly rinsed with distilled water, and again surface liquid was blotted off. To ensure that the central cavity of the coleoptile was drained, each segment was stood endwise on filter paper. The plant material was finally transferred to dry vials and quickly killed and dried by heating in an oven at 90°C for one hour.

To assay the uptake of C^{14}, each lot of segments was divided into batches comprised of 10 segments. Each batch was finely ground in a small agate mortar with a small volume of methanol until uniformly homogenized. 0.6 ml of a 5% solution of glue (Lepage gum) was added to the homogenate, and the pestle and sides of the mortar were washed with small volumes of methanol. The contents of the mortar were gently stirred so as to suspend the plant tissue particles in the gum medium, and the suspension was then transferred, by means of a blunt-nosed pipette, into a 14-mm diameter, weighed aluminium planchet. The planchet was agitated gently so as to spread the suspension uniformly and dried down under an infra-red lamp. It was then

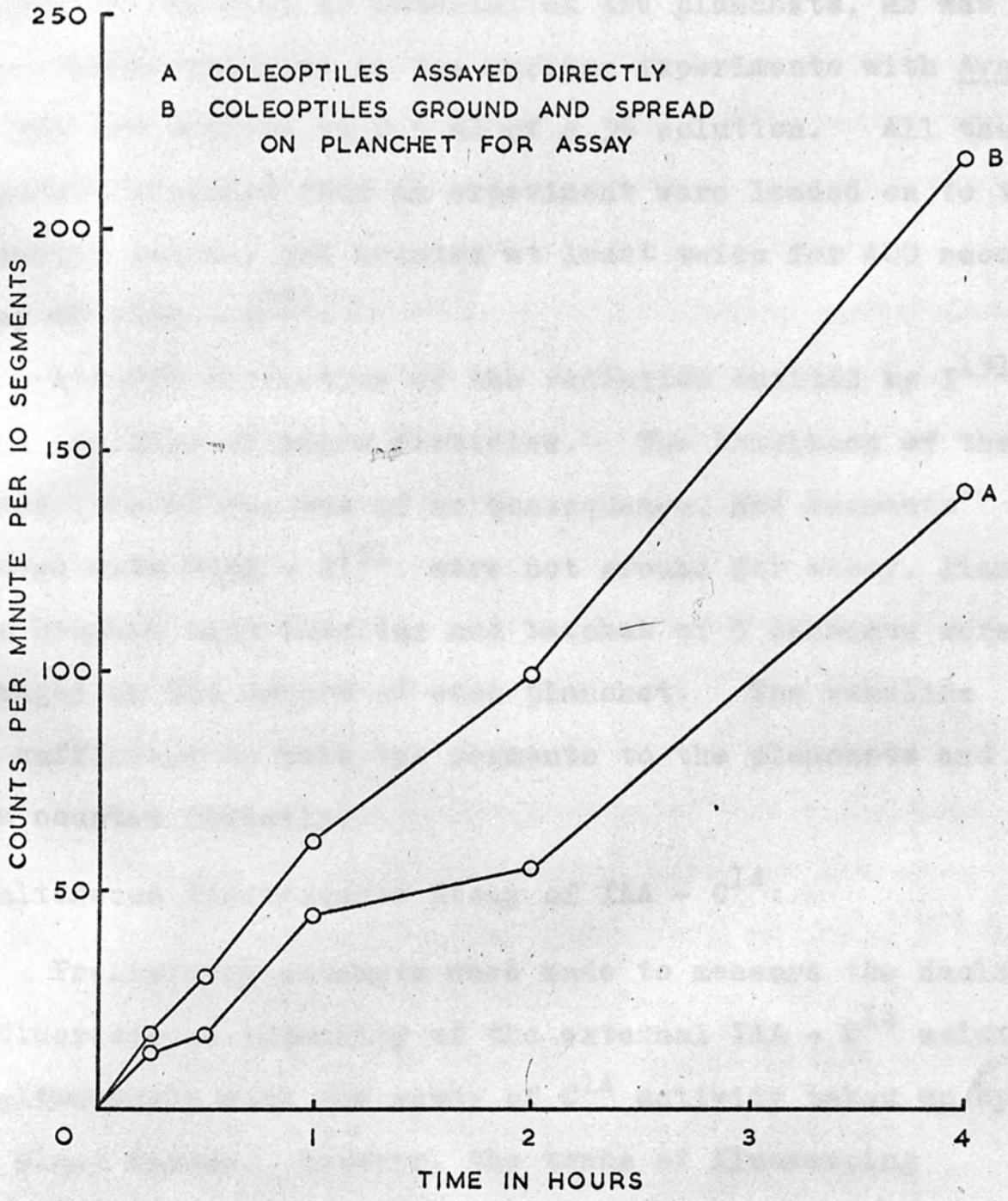
reweighed and counted in the Panax scintillation counter at least two or three times for 100-400 seconds.

Johnson and Bonner (1956) arranged their Avena coleoptile segments, treated with radioactive 2,4-D-C^{14}, on planchets in a monosectional layer and found that a linear relationship existed between the number of segments and the counts obtained. This was attempted in the present work (Curve A, Fig.D), and the sections were then homogenized and counted in the usual manner (curve B, Fig.D). It is evident from the graph that grinding and spreading the plant material considerably increased the efficiency and reproducibility of the assay.

Zea mesocotyl and Phaseolus hypocotyl:

For each treatment, batches of 10 segments (one batch for every interval wherein the treatment was sampled) were each floated on 2.0 ml of the radioactive experimental media in 1" x 1" glass vials. All manipulations were conducted under dim red light, and in between sampling periods the vials were shaken for the duration of the experiment on a mechanical rocker at 25°C in the dark. At periodic intervals, one vial representing each treatment was taken out and the segments removed for assay. Subsequent procedures for preparing the segments for counting were identical with

FIG. D


A COLEOPTILES ASSAYED DIRECTLY
B COLEOPTILES GROUND AND SPREAD ON PLANCHET FOR ASSAY
COUNTS PER MINUTE PER 10 SEGMENTS
250
200
150
100
50
0
1
2
3
4
TIME IN HOURS
A
B

those described for Avena. However, as the tissue segments were more bulky, the 10 segments comprising a sample were divided into two lots of 5 segments each. To prevent flaking of the film of material on the planchets, as was occasionally obtained in the earlier experiments with Avena, the gum was reduced to 0.5 ml of a 3% solution. All the planchets prepared from an experiment were loaded on to the automatic Betamat and counted at least twice for 400 seconds.

Assay of TIBA - I^{131}:

A large proportion of the radiation emitted by I^{131} is in the form of gamma particles. The thickness of the tissue, therefore, was of no consequence, and segments treated with TIBA - I^{131} were not ground for assay. Planchets were brushed with vaseline and batches of 5 segments were arranged on the centre of each planchet. The vaseline was sufficient to hold the segments to the planchets and they were counted directly.

Simultaneous Fluorescence Assay of IAA - C^{14}:

Preliminary attempts were made to measure the decline in fluorescence intensity of the external IAA - C^{14} solution simultaneously with the assay of C^{14} activity taken up by the plant tissue. However, the trace of fluorescing contaminant present in the radioactive IAA solution considerably affected the fluorimetric analysis. The fluctuations in

fluorsecence intensity were considered too erratic to be disregarded and attempts in this direction were, therefore, abandoned.

Treatment of Radioactivity Assay Data:

As the IAA - C^{14} used in the present work was prepared by alkaline hydrolysis of IAN - C^{14}, values for the initial concentrations of IAA in each treatment are based on the assumption of 98% recovery, by virtue of the negligible losses in activity recorded during conversion and extraction of the compound. As mentioned earlier, attempts to determine accurately the actual concentrations of the IAA - C^{14} stock solutions by fluorescence assay proved unsuccessful.

No attempt was made to convert the activity counts, obtained from segments treated with IAA - C^{14}, into absolute amounts of IAA for the following reasons:

(a) the estimate of the concentration of IAA - C^{14} used in the experiments, is an approximation based on the assay of recovery of the compound after hydrolysis of IAN - C^{14} and the subsequent extraction procedures;

(b) an accurate estimate of actual uptake of IAA - C^{14} by the plant tissue would necessitate the application of corrections to the values obtained to account for the degrees of possible oxidative decarboxylation of IAA under the

varying experimental conditions. As the primary purpose of this investigation was to study the pattern of uptake interactions between the growth substances tested, the counts themselves, being representative of concentrations, provide quite adequate data for analysis.

Each sample (representing a single treatment at a specific time interval during an uptake experiment) consisting of 10 segments, was divided into two lots of 5 segments each for activity assay. Each lot was counted twice for 400 seconds. The mean value of the four readings obtained for each sample was calculated and background counts subtracted. These figures were then converted to counts per minute (CPM) and are presented in the data per 5 Zea mesocotyl or Phaseolus hypocotyl segments. In the case of Avena, the data ~~is~~ are presented as CPM per 10 coleoptile segments.

Iodine^{131} has a half-life of only 8.04 days. Therefore, in the case of experiments conducted with TIBA - I^{131}, corrections for decay were applied so as to bring the results of all the experiments to the same level.

EXPERIMENTAL METHODS: (C) SHADOWGRAPH TECHNIQUE FOR MEASURING SEGMENT ELONGATION, ETC.:

Increments in segment length were measured using a shadowgraph technique. A strip of graph paper was affixed to a clear glass plate and the plate was mounted on the holder of a photographic enlarger. The focus was adjusted so that the projected image was magnified by a factor of 3.0. Batches of segments were arranged in lots of 10 or more (representing a treatment) on similar glass plates and shadowgraphs prepared on sheets of Kodak positive photography paper. The prints were developed in Kodak ID 36 developer, and segment length measured to the nearest 0.5 mm. The figures presented in the Tables represent the mean segment length described by each sample.

Methods employed for chromatographic and autoradiographic analysis of plant material, and for measurements of respiratory decarboxylation of IAA - C^{14}, have been described in detail in the relevant sections of the chapter entitled "Results" .

ANALYSIS OF UPTAKE DATA:

For all apparently linear relationships obtained in the data, the linear regressions were calculated by the method of least squares (Snedecor, 1940; Moroney, 1951:

Bailey, 1959). It was found, as will be described in detail in the next chapter, that steady state uptake, representing the metabolic phase, was attained after one hour of incubation and maintained thereafter for the duration of the experiment. The rate of uptake was therefore calculated generally from the values for uptake obtained during this period (one hour onwards) so as to represent the phase of active absorption. Rate is represented by the regression coefficient of the curve obtained when uptake is plotted against time. In those cases where rate has been calculated from the start of the experiment (0 Hours), this has been clearly pointed out in the data with reasons. In such instances, the values for rate of uptake calculated from 1 hour have also been included in the tables for the sake of comparison, wherever it was considered relevant. The degree of inhibition or stimulation of uptake, as a consequence of the various interactions between the growth substances tested, has been expressed in the results on the basis of the percentage difference between the rates of uptake exhibited by control and treated samples.

Presentation of Data:

The data have been presented wherever possible in graphical form. The graphs are supported by detailed tables

included in the appendix. As exponential curves were rarely obtained, all the figures have been plotted on linear scales. Where experiments were replicated, values for mean deviation have been included in the tables, and where possible, have been represented by vertical bars on the relevant graphs. Tables in the appendix have been numbered in Roman numerals and references to them in the running matter of the text are always in parenthesis.

RESULTS

RESULTS

I (a)

MATERIAL: *Avena* coleoptile

METHOD: Fluorescence assay

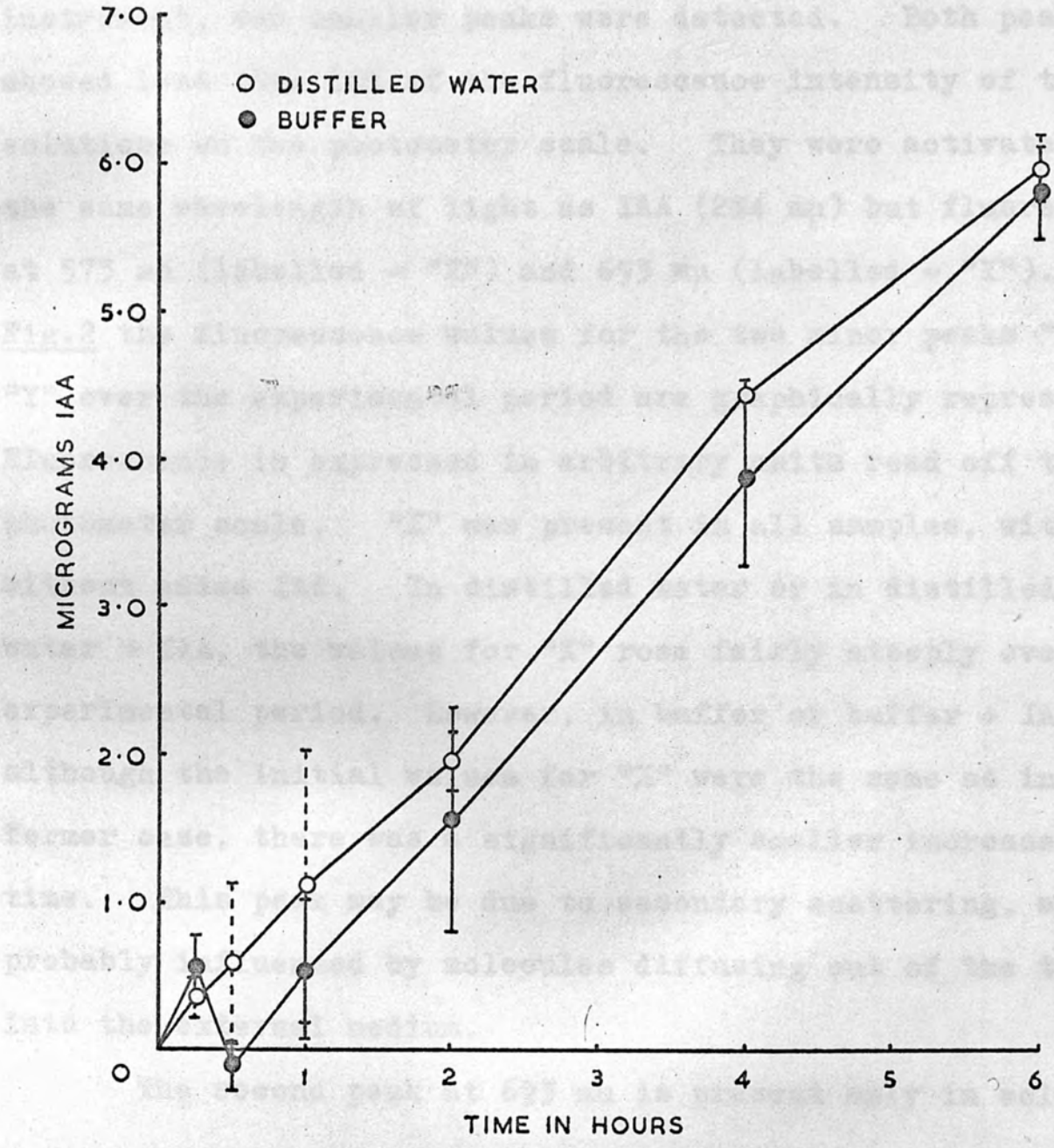
GROWTH-SUBSTANCE: IAA

The initial experiments, using *Avena* coleoptile segments, were planned towards (a) examining the rates of uptake of IAA from distilled water and buffer mediums respectively, and (b) checking on endogenous fluorescing compounds that might possibly diffuse out of the tissue into the experimental solutions and interfere with the assay of IAA.

In Fig.1 the mean values for uptake from two experiments are plotted against time. It is evident that there is no significant difference in the uptake of IAA from distilled water or buffer solution. It may also be seen that whereas uptake from 1 hour is a linear function of time, the initial values for uptake over the first hour vary considerably. (The data is presented in Table I). All subsequent uptake experiments were conducted with the growth substances made up to the desired concentrations in the KH_2PO_4 - citric acid buffer at pH 5.0.

When coleoptile segments were floated on distilled

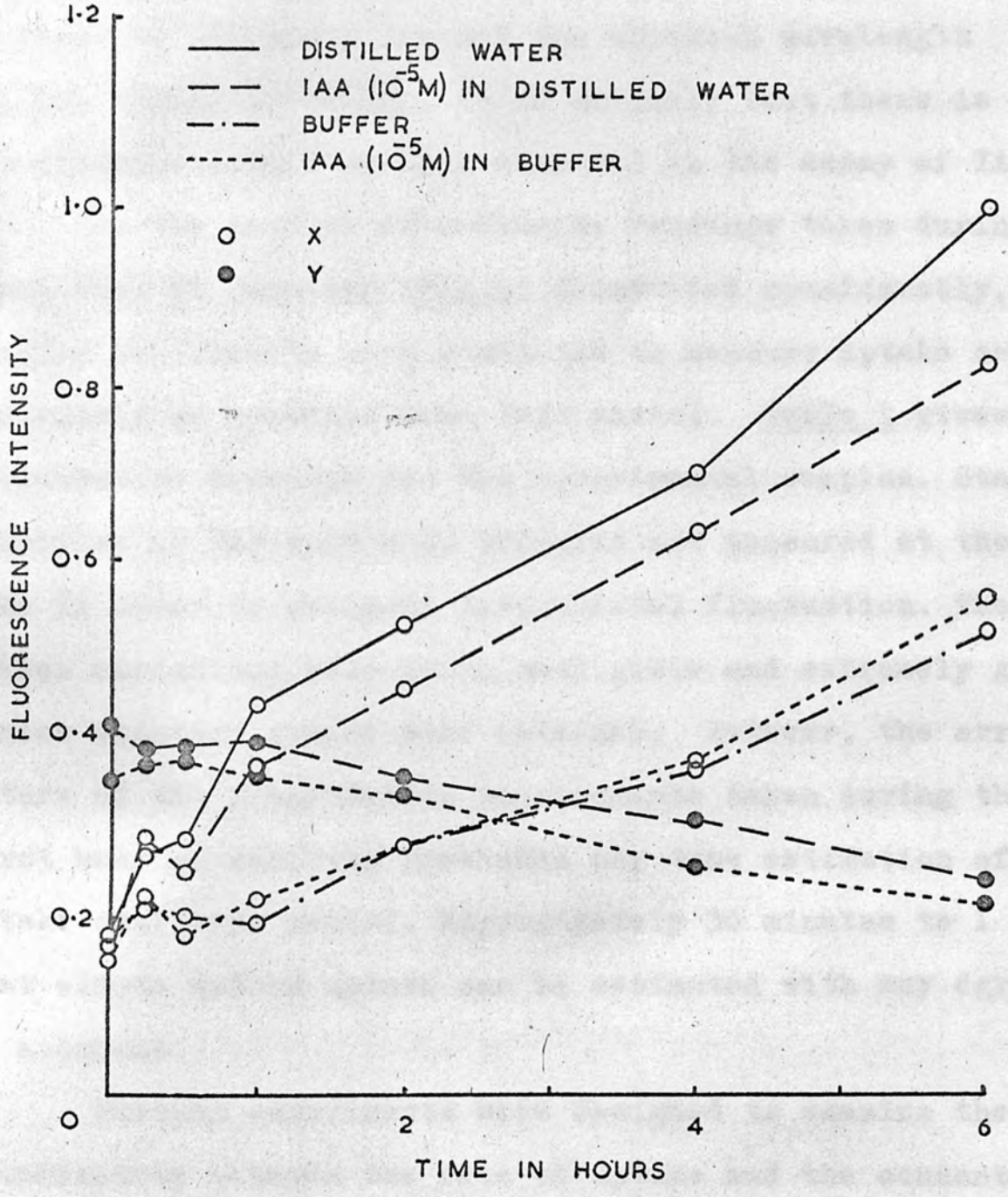
FIG. 1: UPTAKE OF IAA



water or buffer solution in the absence of IAA, there was no detectable diffusion from the tissue of any compounds with the same fluorescent properties as IAA. On scanning the solutions for fluorescence over the wavelength range of the instrument, two smaller peaks were detected. Both peaks showed less than 10% of the fluorescence intensity of the IAA solutions on the photometer scale. They were activated at the same wavelength of light as IAA (284 mμ) but fluoresced at 573 mμ (labelled - "X") and 693 mμ (labelled - "Y"). In Fig.2 the fluorescence values for the two minor peaks "X" and "Y" over the experimental period are graphically represented. Fluorescence is expressed in arbitrary units read off the photometer scale. "X" was present in all samples, with or without added IAA. In distilled water or in distilled water + IAA, the values for "X" rose fairly steeply over the experimental period. However, in buffer or buffer + IAA, although the initial values for "X" were the same as in the former case, there was a significantly smaller increase with time. This peak may be due to secondary scattering, and is probably influenced by molecules diffusing out of the tissue into the external medium.

The second peak at 693 mu is present only in solutions

FIG. 2


DISTILLED WATER
IAA (10^{-5}M) IN DISTILLED WATER
BUFFER
IAA (10^{-5}M) IN BUFFER
X
Y
FLUORESCENCE INTENSITY
1·2
1·0
0·8
0·6
0·4
0·2
0
2
4
6
TIME IN HOURS

of IAA, in water or in buffer, and declines in intensity over the experimental period at about the same rate as IAA. This peak is probably due to a contaminant present in the synthetic IAA used, but, as the fluorescence intensity of this compound is extremely low and the emission wavelength maximum widely differing, it is unlikely that there is any interference caused by this compound in the assay of IAA.

As the initial fluorescence readings taken during the first hour of sampling (Fig.1) fluctuated considerably, two further experiments were conducted to measure uptake as accurately as possible over this period. Table 1 gives the fluorimetric readings for the experimental samples. Standard solutions of IAA were also prepared and measured at the same time in order to evaluate instrumental fluctuation. The latter variations were quite negligible and extremely good linear standard graphs were obtained. However, the erratic nature of the fluorimetric measurements taken during the first hour of sampling precluded any true estimation of IAA uptake over this period. Approximately 30 minutes to 1 hour must elapse before uptake can be estimated with any dgreee of accuracy.

Further experiments were designed to examine the relationship between the rate of uptake and the concentration

TABLE 1

Time of Sampling	Fluorescence Intensity	
	Expt. 1	Expt. 2
In. (a)	60.0	49.3
In. (b)	63.0	52.4
5 min.	60.0	52.3
10 min.	63.8	54.3
15 min.	61.5	53.1
30 min.	63.0	52.0
1 hour	60.0	51.0

'Initial phase' of IAA uptake represented in terms of decreasing fluorescence intensity. 100 Avena coleoptile segments per 10 ml of 10^{-5}M IAA.

In. (a): Sample taken from the solution just prior to adding the tissue segments.

In. (b): Sample taken immediately after introducing the segments into the test solution.

of IAA in the external medium over a period of 4 hours. The mean uptake values from two experiments, in which varying concentrations of IAA (5×10^{-6}M - 5×10^{-5}M) were tested, are graphically presented in Fig.3 (Data: Table II). As previously noted there was no clear pattern of uptake over the first hour with all concentrations of IAA applied. The fluctuations in the early readings were more pronounced with the lower concentrations of IAA. However, uptake from 1-4 hours was fairly linear for all concentrations, some slight decrease in the slopes of the curves being evident with time. In Fig.4, rate of uptake of IAA is plotted as a function of concentration. A linear curve is obtained - i.e. uptake was directly proportional to external concentration over the range examined. (Data: Table III)

In the technique of assay employed, each Petri dish was sampled, five or six times, during the course of an experiment, to measure the fall in IAA concentration. As it was feared that the consequent changing ratios of volume of tissue to volume of external solution might affect uptake of IAA by the segments, an experiment was conducted towards examining any possible effect due to sampling. Five Petri dishes, A to E, each containing 10 ml of 10^{-5}M IAA and 100 coleoptile segments were prepared, and the sampling staggered so that at the first sampling interval only A was sampled;

FIG. 3: UPTAKE OF IAA

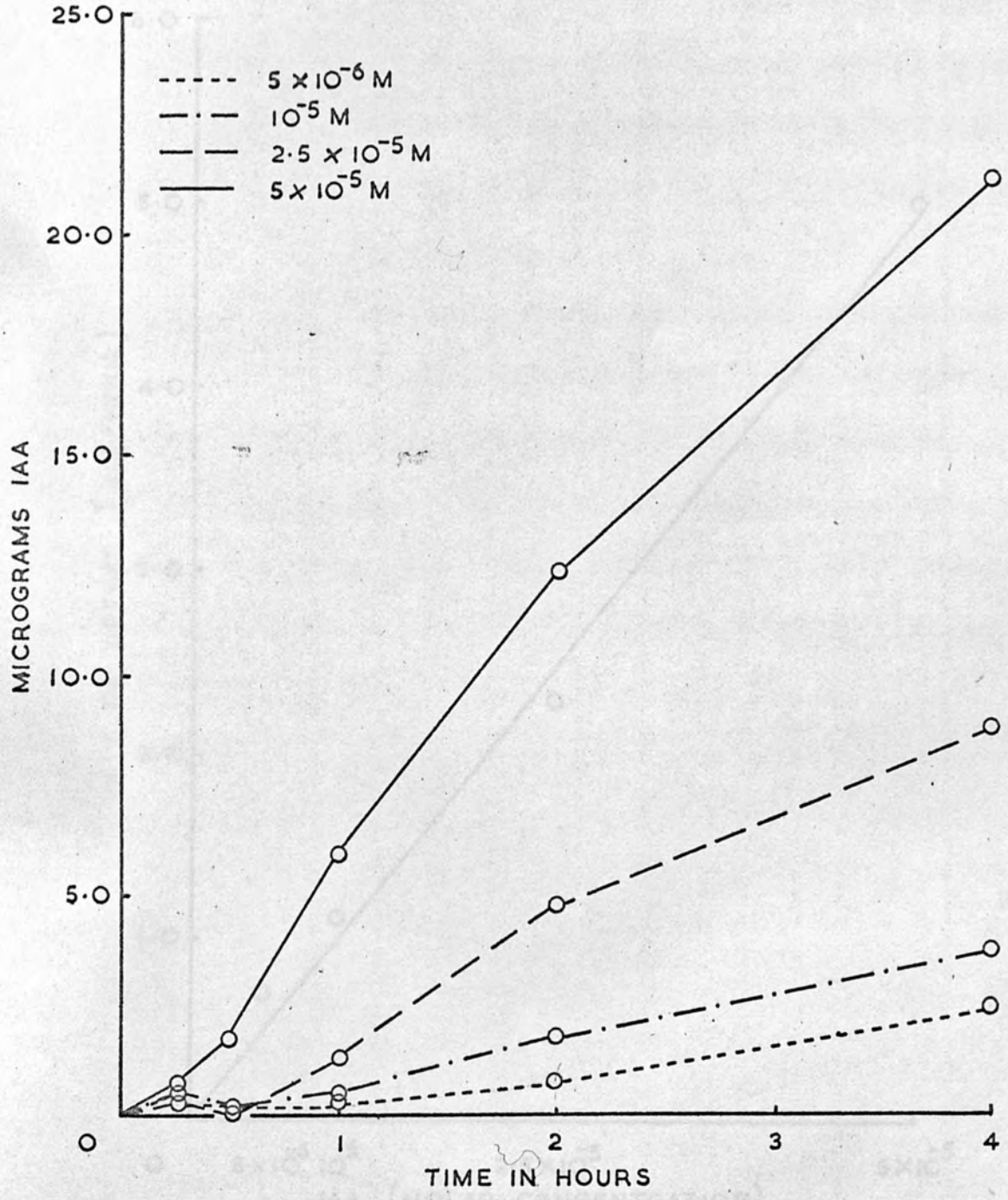
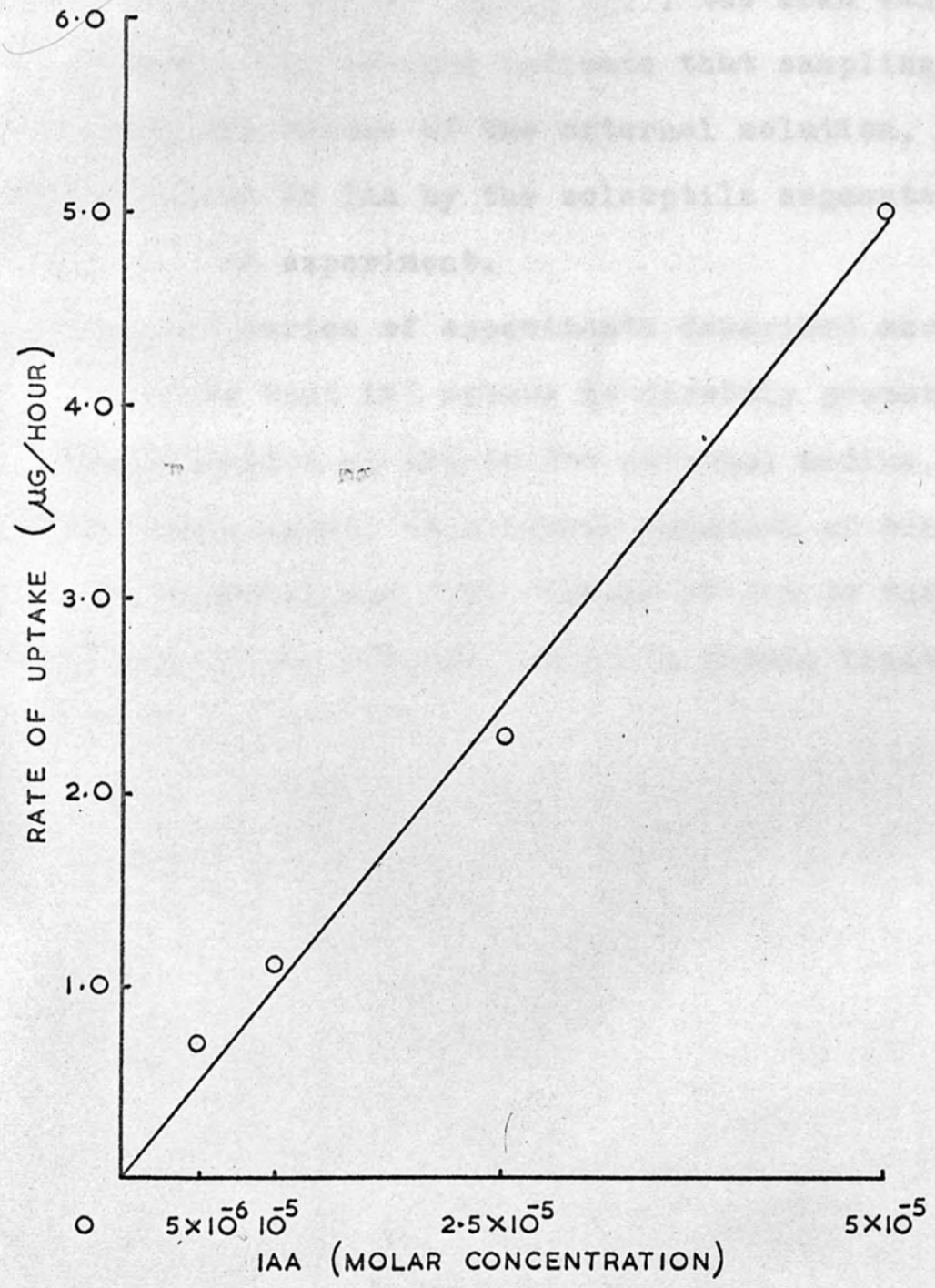


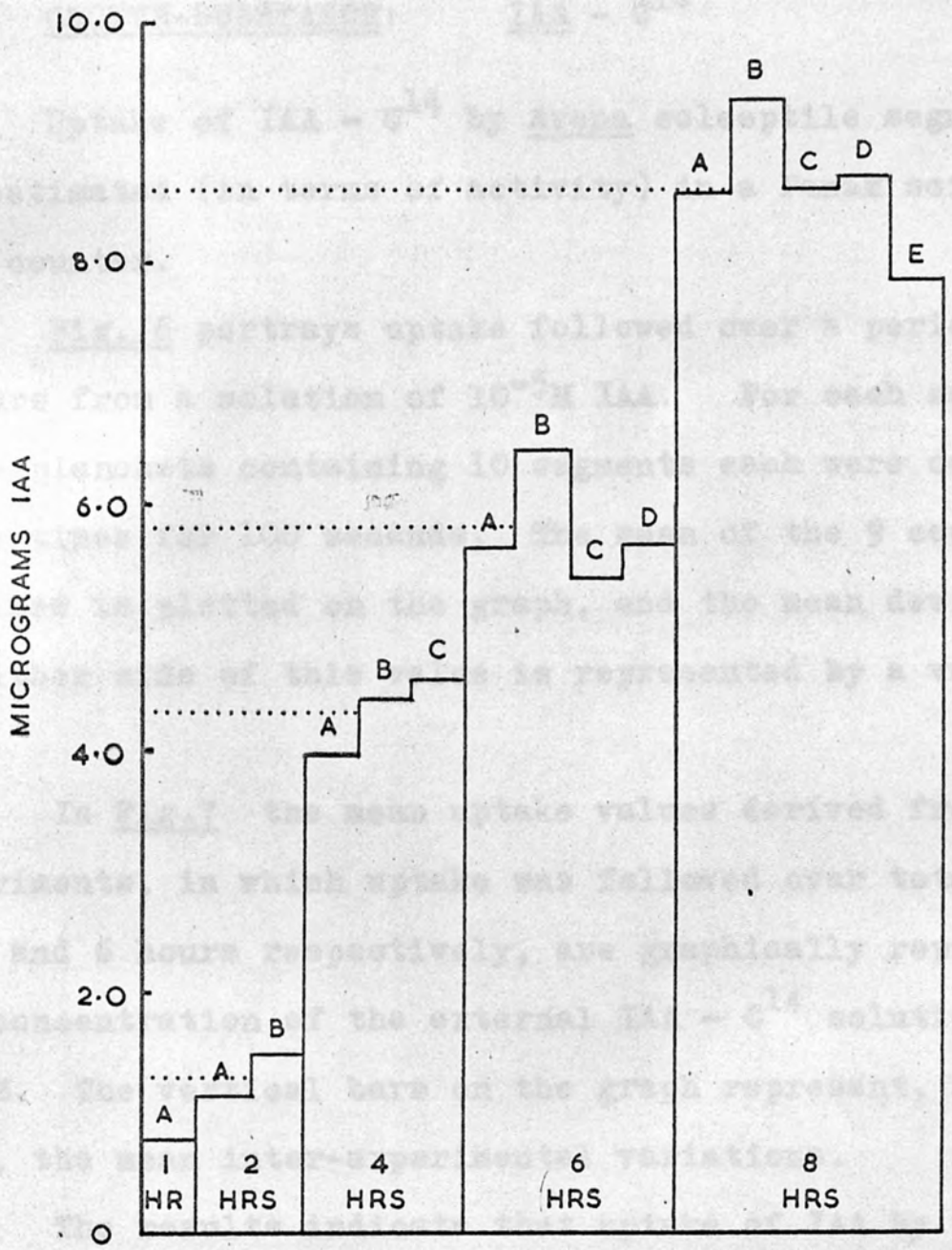
FIG. 4


6·0
5·0
4·0
3·0
2·0
1·0
RATE OF UPTAKE (µG/HOUR)
0
5×10⁻⁶
10⁻⁵
2·5×10⁻⁵
5×10⁻⁵
IAA (MOLAR CONCENTRATION)

at the second interval, A and B were sampled; at the third, A, B and C; and so on over an 8-hour period. The uptake figures obtained for each lot are plotted in the form of a histogram in Fig.5 ,(Data: Table IV) , the mean values being included. The results indicate that sampling, and thus reducing the volume of the external solution, does not affect the uptake of IAA by the coleoptile segments within the limits of the experiment.

From the series of experiments described above it may be concluded that (a) uptake is directly propotional to the concentration of IAA in the external medium, (b) steady state uptake is a linear function of time over a period of 8 hours, and (c) uptake of IAA is independent of the volume of the external solution within limits.

FIG. 5: EFFECT OF SAMPLING ON IAA UPTAKE



I (b)

MATERIAL: *Avena* coleoptile

METHOD: Radioactivity assay

GROWTH-SUBSTANCE: IAA - C^{14}

Uptake of IAA - C^{14} by *Avena* coleoptile segments was estimated (in terms of activity) in a Panax scintillation counter.

Fig. 6 portrays uptake followed over a period of 2 hours from a solution of 10^{-5}M IAA. For each sample, three planchets containing 10 segments each were counted three times for 100 seconds. The mean of the 9 counts thus obtained is plotted on the graph, and the mean deviation on either side of this value is represented by a vertical bar.

In Fig.7 the mean uptake values derived from two experiments, in which uptake was followed over total periods of 4 and 6 hours respectively, are graphically represented. The concentration of the external IAA - C^{14} solution was 10^{-5}M. The vertical bars on the graph represent, in this case, the mean inter-experimental variations.

The results indicate that uptake of IAA by the coleoptile segments is a linear function of time throughout

FIG. 6: UPTAKE OF IAA-C^{14}

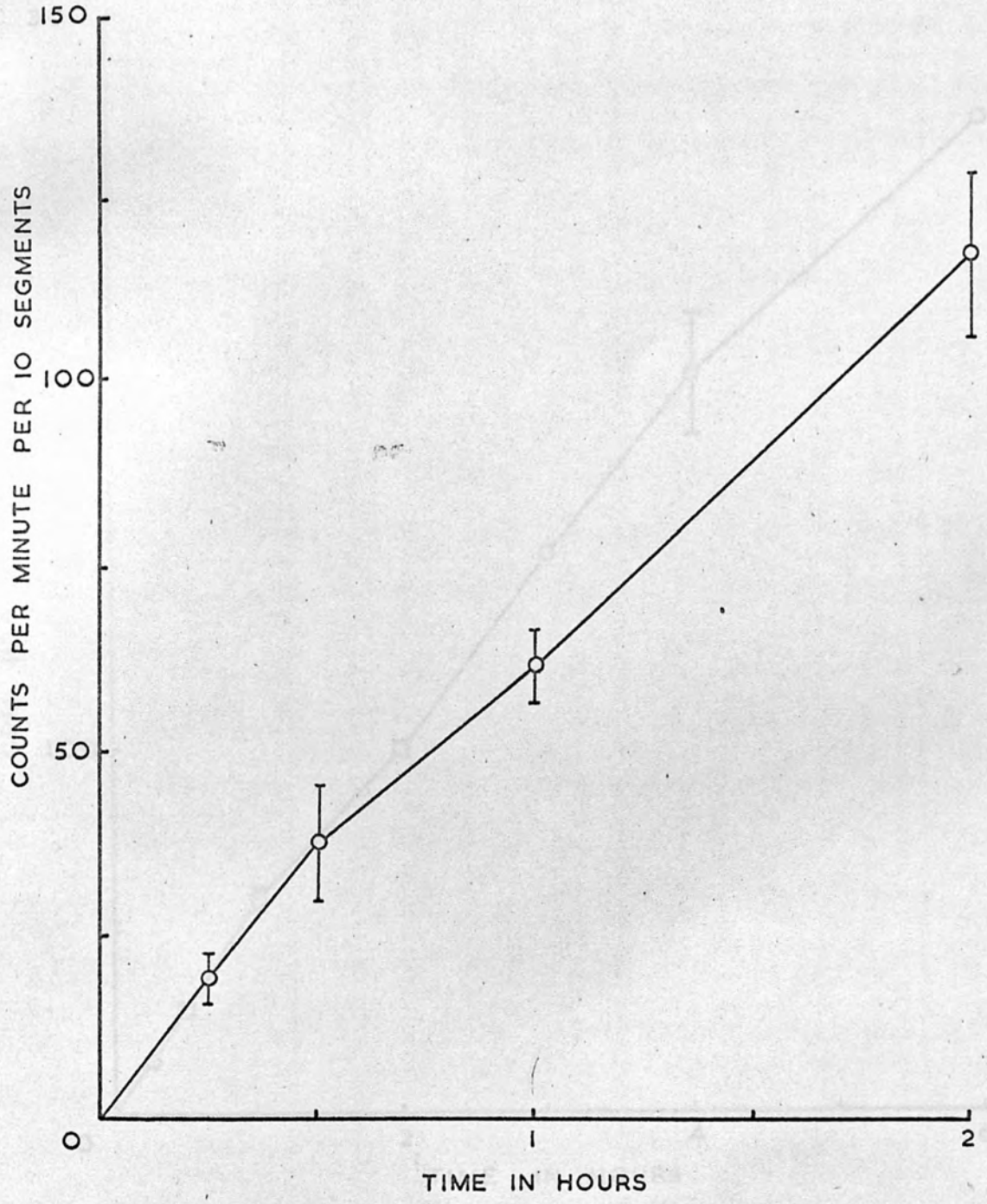
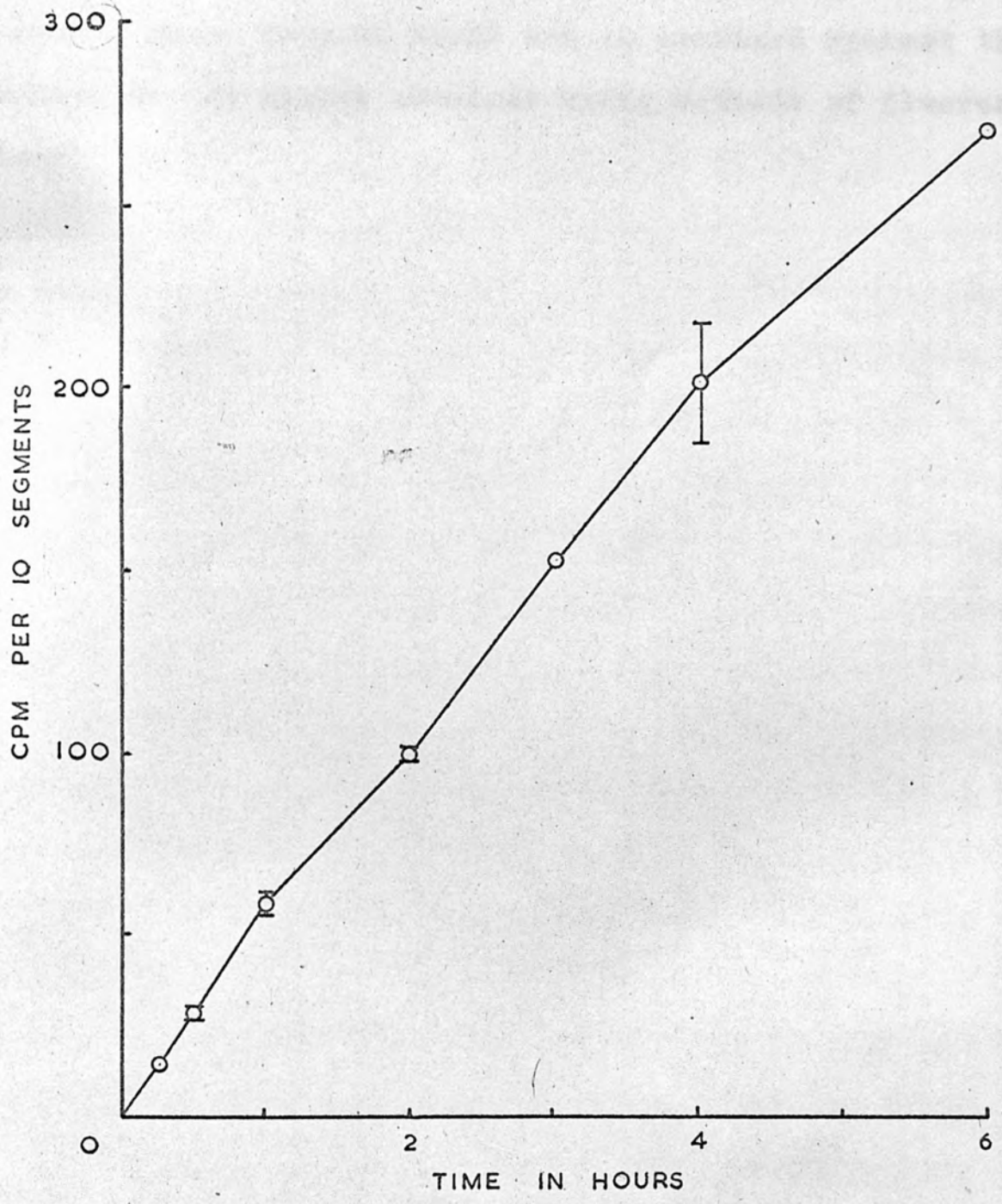


FIG. 7: UPTAKE OF IAA-C^{14}



the experimental period; so much so that no clear distinction could be made between any initial physical phase of uptake and subsequent steady state uptake. In this respect, these results stand out in contrast against the pattern of IAA uptake obtained using methods of fluorescence assay.

II

MATERIAL: *Avena* coleoptile

METHOD: Fluorescence assay

GROWTH-SUBSTANCES: IAA + NaCN

The results of the preliminary uptake experiments employing fluorescence assay described in section I (a) indicate that a steady state of uptake, presumably metabolic, is attained after approximately one hour of incubation in the IAA medium. In an attempt to separate this phase of uptake from an initial phase of IAA absorption due to physical factors, the segments were pretreated, for one hour after cutting, with sodium cyanide solutions ranging in concentration from 10^{-5}M to 10^{-3}M. Control batches of segments were equilibrated in buffer during this period. At the end of one hour the treated segments were removed, superficially blotted on filter paper, briefly rinsed in buffer and transferred to solutions of IAA at a concentration of 10^{-5}M. Uptake was subsequently measured over a four hour period.

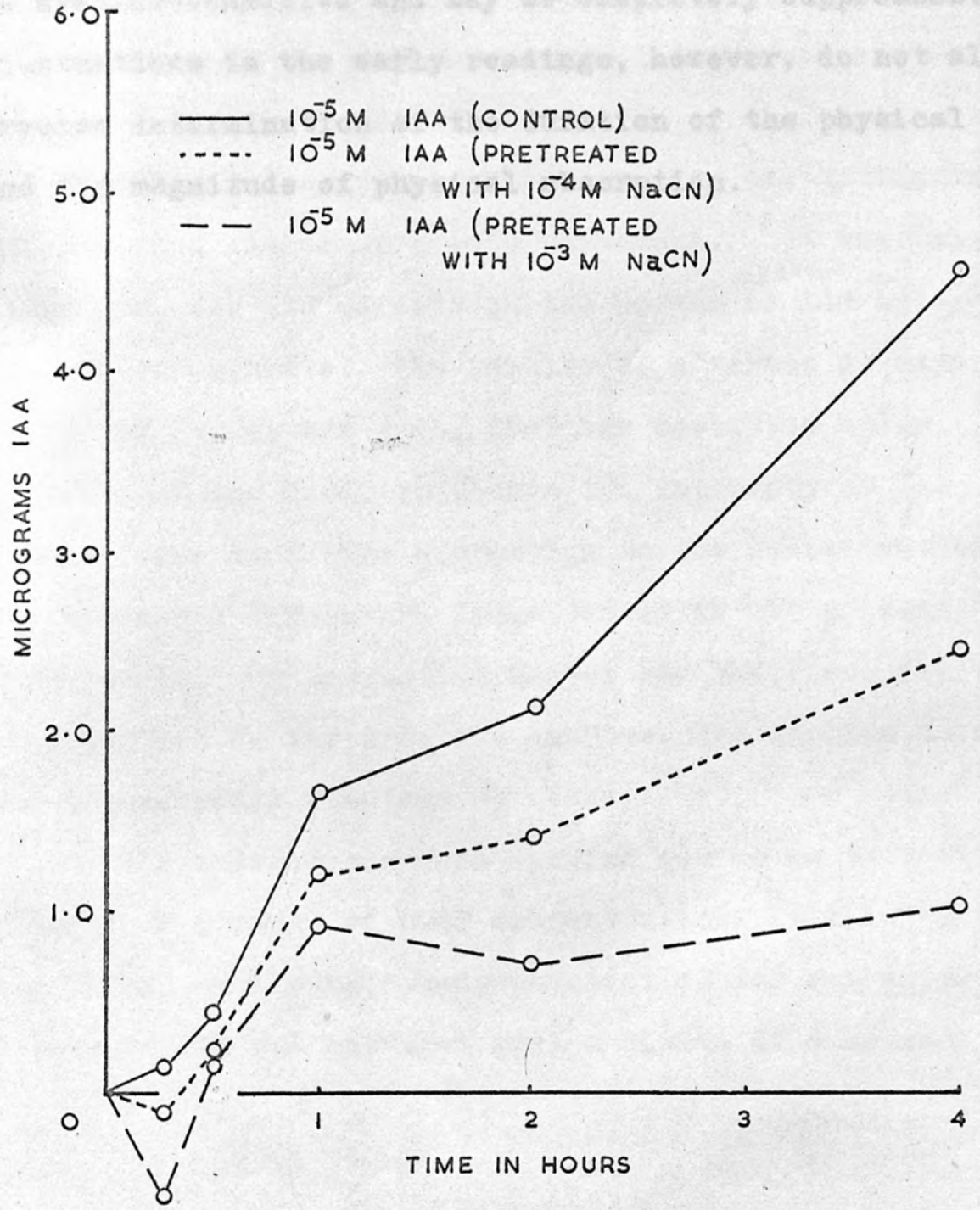
In the first experiment, pretreatment with 10^{-5}M and 10^{-4}M solutions of NaCN was tried. At the lower concentration, cyanide appeared to have no inhibitory effect at all on the rate of IAA uptake. At 10^{-4}M there was a slight depression of the rate of uptake amounting to 5.9%.

This experiment was repeated, whereupon a slight inhibition (4.14%) of the rate of uptake was obtained with 10^{-5}M NaCN, and inhibition amounting to 9.39% with the higher concentration of cyanide. However, the initial fluorescence figures (15m, 30m, 1 hour), and consequently the calculated uptake values, for control and treated batches vary so widely that when slopes for rate of uptake were calculated from the start of the experiment (0 hour) inhibition was as much as 26% with 10^{-4}M NaCN and absent with 10^{-5}M NaCN. (Data: Table V).

In subsequent experiments the concentration of NaCN was stepped up to 10^{-3}M and the treated segments were allowed to equilibrate in buffer for 30 minutes before being transferred to the IAA medium. Two replicate experiments showed fairly close agreement, In Fig. 8 the mean uptake values are plotted against time. (Data: Table VI). Uptake after one hour was almost completely inhibited by NaCN at 10^{-3}M, while at 10^{-4}M there was considerable depression of uptake rate, although the degree of inhibition varied somewhat between experiments.

The results suggest the existence of two phases in the uptake system. The initial phase, with a duration of approximately 1 hour, may represent the sum of passive and

FIG. 8


6·0
5·0
4·0
3·0
2·0
1·0
0
MICROGRAMS IAA
10^{-5} M IAA (CONTROL)
10^{-5} M IAA (PRETREATED WITH 10^{-4}M NaCN)
10^{-5} M IAA (PRETREATED WITH 10^{-3} M NaCN)
1
2
3
4
TIME IN HOURS

active uptake processes. Cyanide pretreatment affects this phase to some extent, in so far as it presumably inhibits active uptake. Subsequent absorption of IAA (after 1 hour) is cyanide-sensitive and may be completely suppressed. Fluctuations in the early readings, however, do not allow a precise determination of the duration of the physical phase, and the magnitude of physical absorption.

III

MATERIAL: *Avena* coleoptile

METHOD: Fluorescence assay.

GROWTH-SUBSTANCES: IAA + (N - 1 - naphthyl-methyl - sulphide) - propionic acid (NMSP).

NMSP is a compound well known for its antiauxin effects in a number of growth phenomena. It was tested, therefore, for its effects on the uptake of IAA by *Avena* coleoptile segments. The results of a series of experiments conducted in May and June, 1963 are described below.

NMSP was found to quench the intensity of IAA fluorescence in direct proportion to the concentration of the antiauxin within the range tested in the present experiments. A correction factor was therefore derived, as described in the previous chapter, and applied to all the fluorescence readings obtained.

The experiments were carried out so as to test the effects of a range of NMSP concentrations (5×10^{-6}M to 5×10^{-5}M) on a single concentration of IAA per experiment. Uptake of IAA was measured over a period of 8 hours.

Experiment 1.

A. IAA (5×10^{-6}M) Control.

B. IAA " + NMSP (5×10^{-6}M)

C. IAA " + NMSP (10^{-5}M)

D. IAA " + NMSP (2.5×10^{-5}M)

E. IAA " + NMSP (5×10^{-5}M)

Data: Fig. 9 (Table VII)

Some considerable inhibition of uptake was recorded, the degree declining with decreasing concentrations of NMSP. The experiment was repeated to confirm the reproducibility of this inhibition.

Experiment 2.

A. IAA (5×10^{-6}M) Control.

B. IAA " + NMSP (5×10^{-6}M)

C. IAA " + NMSP (10^{-5}M)

D. IAA " + NMSP (2.5×10^{-5}M)

E. IAA " + NMSP (5×10^{-5}M)

Data: Fig.10 (Table VIII)

The results obtained agree fairly closely with those of the previous experiment.

It was also considered desirable at this point to check the effect that NMSP may have on the growth of the coleoptile segments in IAA. A further batch of 100 segments was floated on buffer medium for the duration of the experiment. At the end of the uptake experiment (8 hours), the six lots of segments, controls and treated, were randomly sampled and segment length measured by the shadowgraph technique. The individual growth measurements

FIG. 9

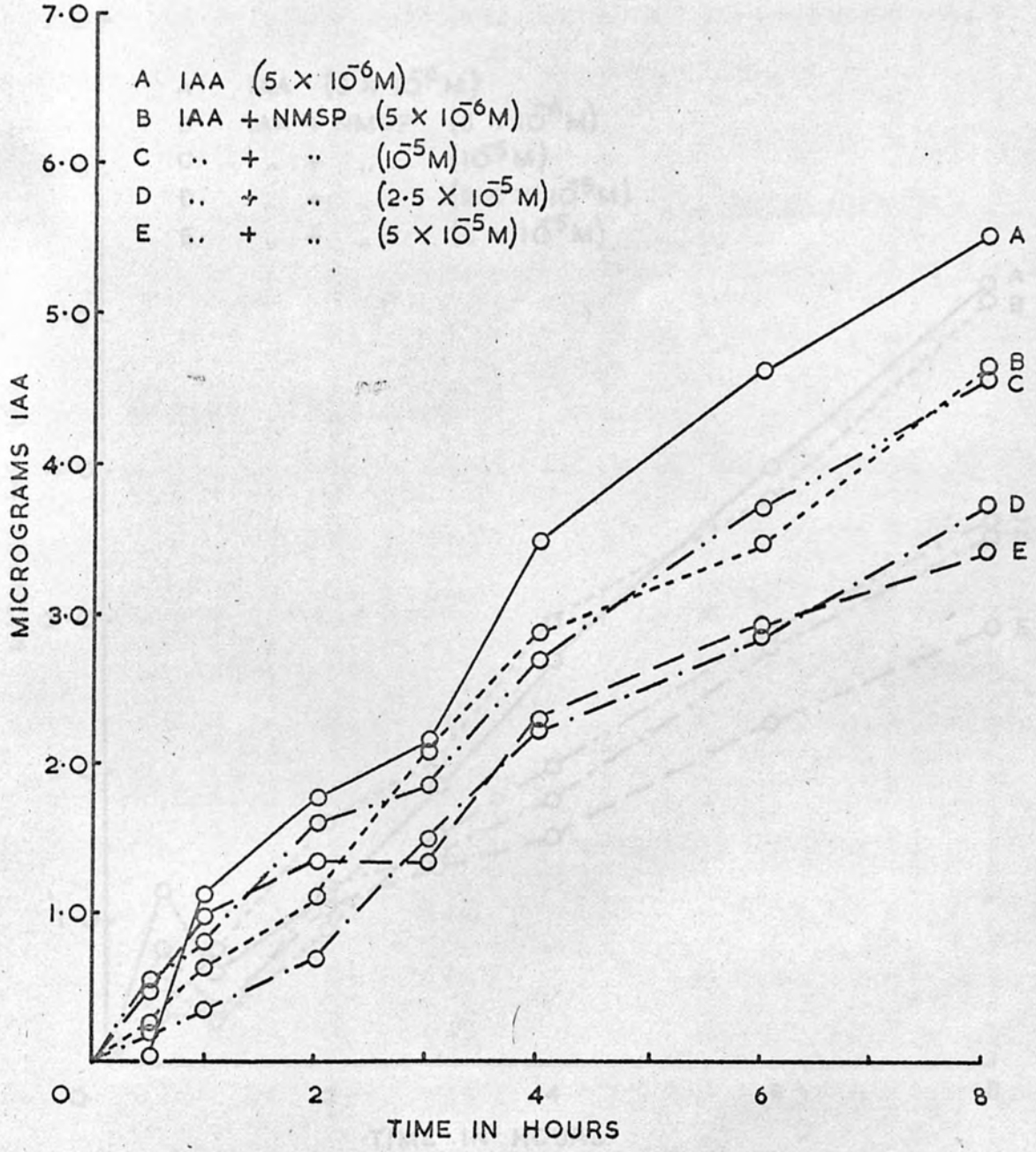
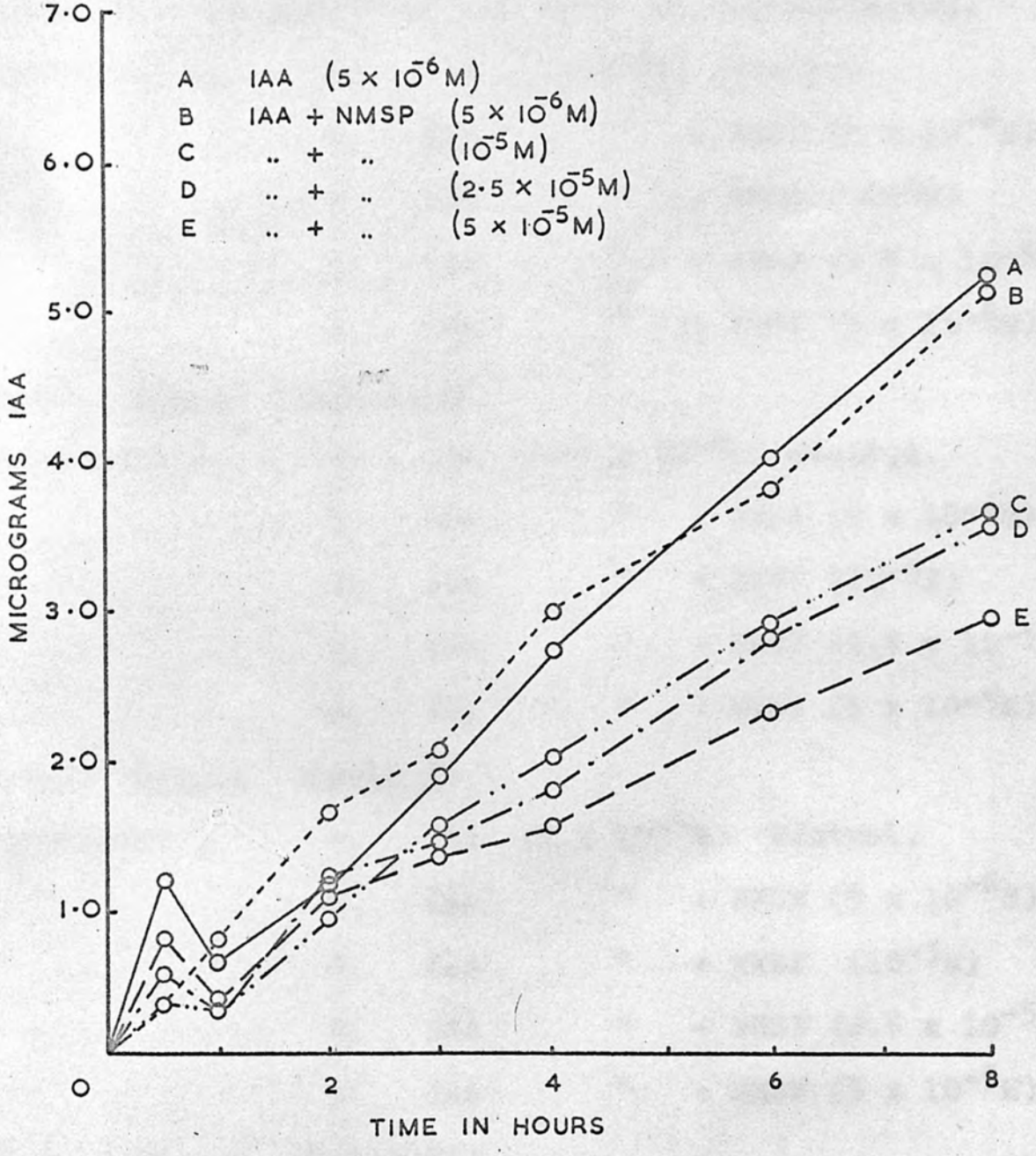

A IAA (5 × 10⁻⁶M)
B IAA + NMSP (5 × 10⁻⁶M)
C .. + .. (10⁻⁵M)
D .. + .. (2·5 × 10⁻⁵M)
E .. + .. (5 × 10⁻⁵M)
MICROGRAMS IAA
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0
2
4
6
8
TIME IN HOURS
A
B
C
D
E

FIG. 10


A IAA (5 × 10⁻⁶M)
B IAA + NMSP (5 × 10⁻⁶M)
C .. + .. (10⁻⁵M)
D .. + .. (2·5 × 10⁻⁵M)
E .. + .. (5 × 10⁻⁵M)
MICROGRAMS IAA
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0
2
4
6
8
TIME IN HOURS
A
B
C
D
E

and mean values are shown in Table 2. There was considerable growth of the control lot, evidenced by all the samples treated with IAA in the external solution. The addition of NMSP had no significant inhibitory or stimulatory effect on growth at any level of concentration.

Experiment 3.
A. IAA (10^{-5}M) Control.
B. IAA " + NMSP (5×10^{-6}M)
C. IAA " + NMSP (10^{-5}M)
D. IAA " + NMSP (2.5×10^{-5}M)
E. IAA " + NMSP (5×10^{-5}M)

Data: Fig.11 (Table IX)

Experiment 4.
A. IAA (2.5×10^{-5}M) Control.
B. IAA " + NMSP (5×10^{-6}M)
C. IAA " + NMSP (10^{-5}M)
D. IAA " + NMSP (2.5×10^{-5}M)
E. IAA " + NMSP (5×10^{-5}M)

Data: Fig.12 (Table X)

Experiment 5.
A. IAA (5×10^{-5}M) Control.
B. IAA " + NMSP (5×10^{-6}M)
C. IAA " + NMSP (10^{-5}M)
D. IAA " + NMSP (2.5×10^{-5}M)
E. IAA " + NMSP (5×10^{-5}M)

Data: Fig.13 (Table XI)

The overall pattern provided by the above experiments

TABLE 2

MEDIA	LENGTH IN CMS X 3												MEAN
	1	2	3	4	5	6	7	8	9	10	11	12	LENGTH
Buffer	3.2	3.4	3.3	3.2	3.3	3.2	3.3	3.3	3.2	3.5	3.3	3.3	3.29
IAA (5×10^{-6}M)	4.1	4.0	4.1	3.8	3.8	3.9	4.2	4.0	3.9	3.8	3.7	4.3	3.97
IAA + NMSP (5×10^{-6}M)	4.1	3.8	3.8	3.7	3.7	3.8	4.0	4.2	3.8	3.8	3.9	___	3.87
IAA + NMSP (10^{-5}M)	3.8	3.8	4.0	3.7	3.8	3.7	4.3	3.7	3.9	3.8	3.9	___	3.85
IAA + NMSP (2.5×10^{-5}M)	3.8	3.6	3.7	3.7	3.8	3.7	3.7	3.8	3.7	3.8	3.7	___	3.73
IAA + NMSP (5×10^{-5}M)	3.8	4.0	4.0	4.0	4.4	4.2	4.0	4.1	3.5	3.9	3.8	___	3.97

Length of Avena coleoptile segments incubated for 8 hours in IAA (5×10^{-6}M) in the absence or presence of NMSP (5×10^{-6}M - 5×10^{-5}M).

FIG. 11

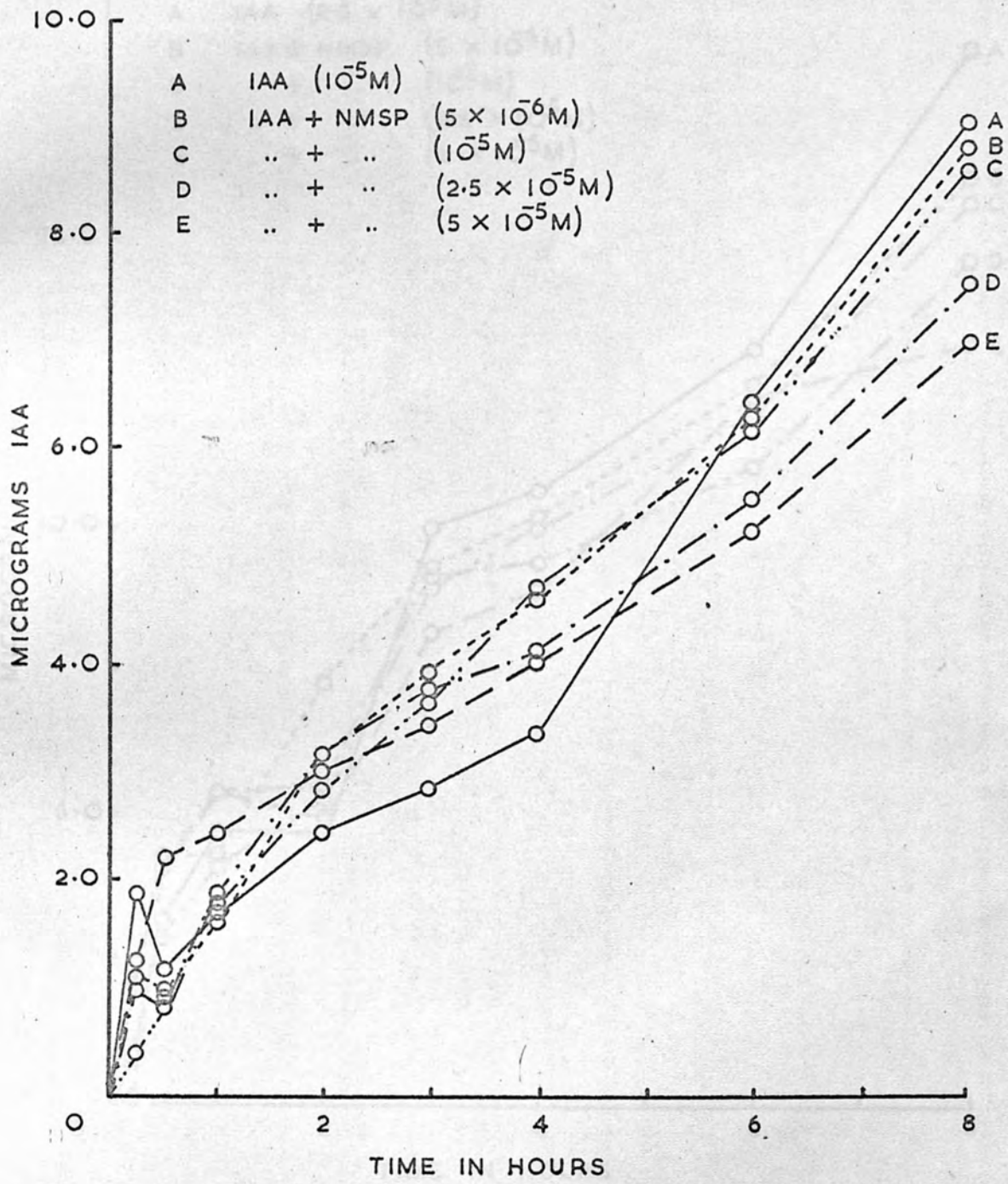

10·0
8·0
6·0
4·0
2·0
0
2
4
6
8
MICROGRAMS IAA
TIME IN HOURS
A IAA (10⁻⁵M)
B IAA + NMSP (5 × 10⁻⁶M)
C .. + .. (10⁻⁵M)
D .. + .. (2·5 × 10⁻⁵M)
E .. + .. (5 × 10⁻⁵M)
A
B
C
D
E

FIG. 12

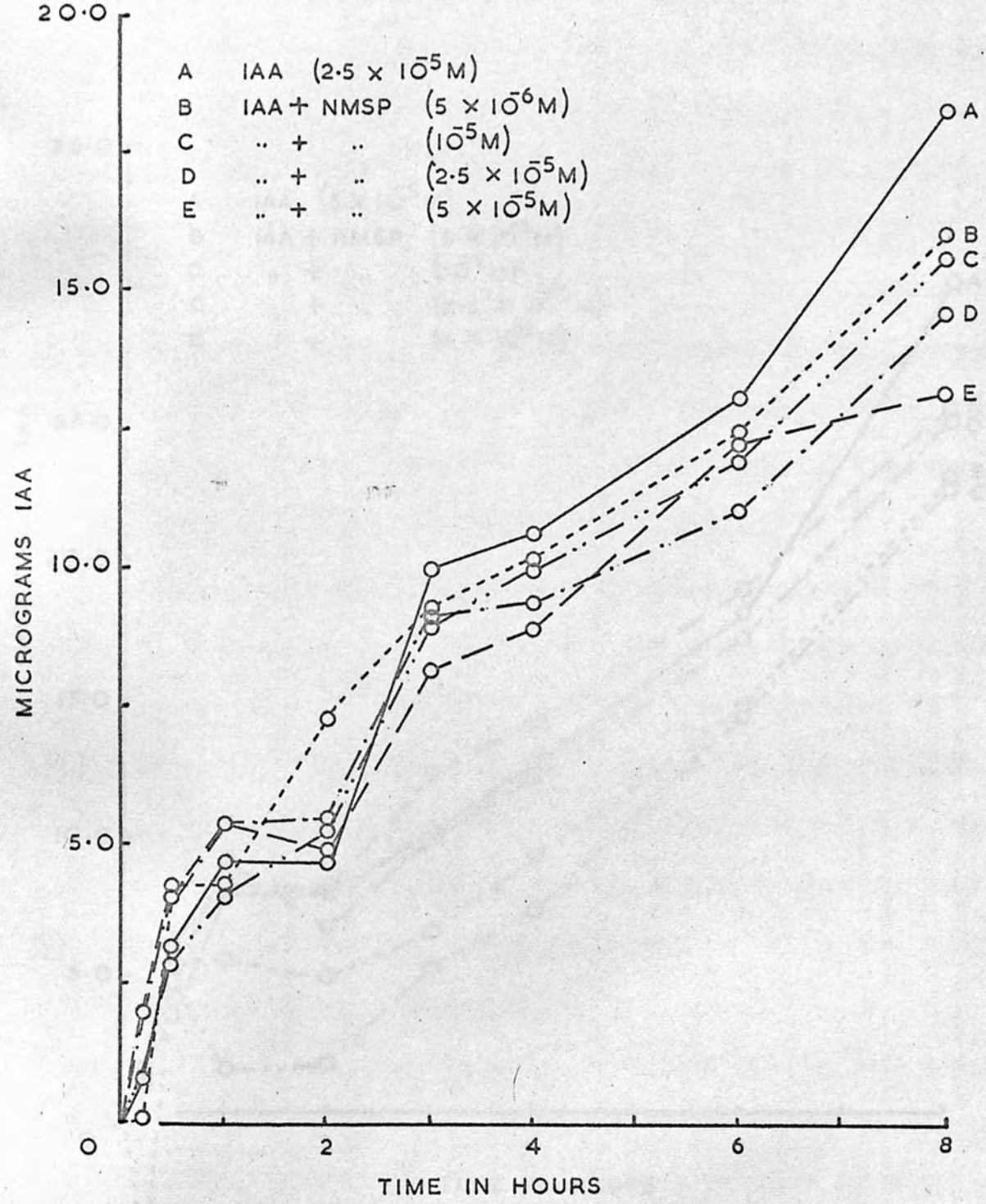
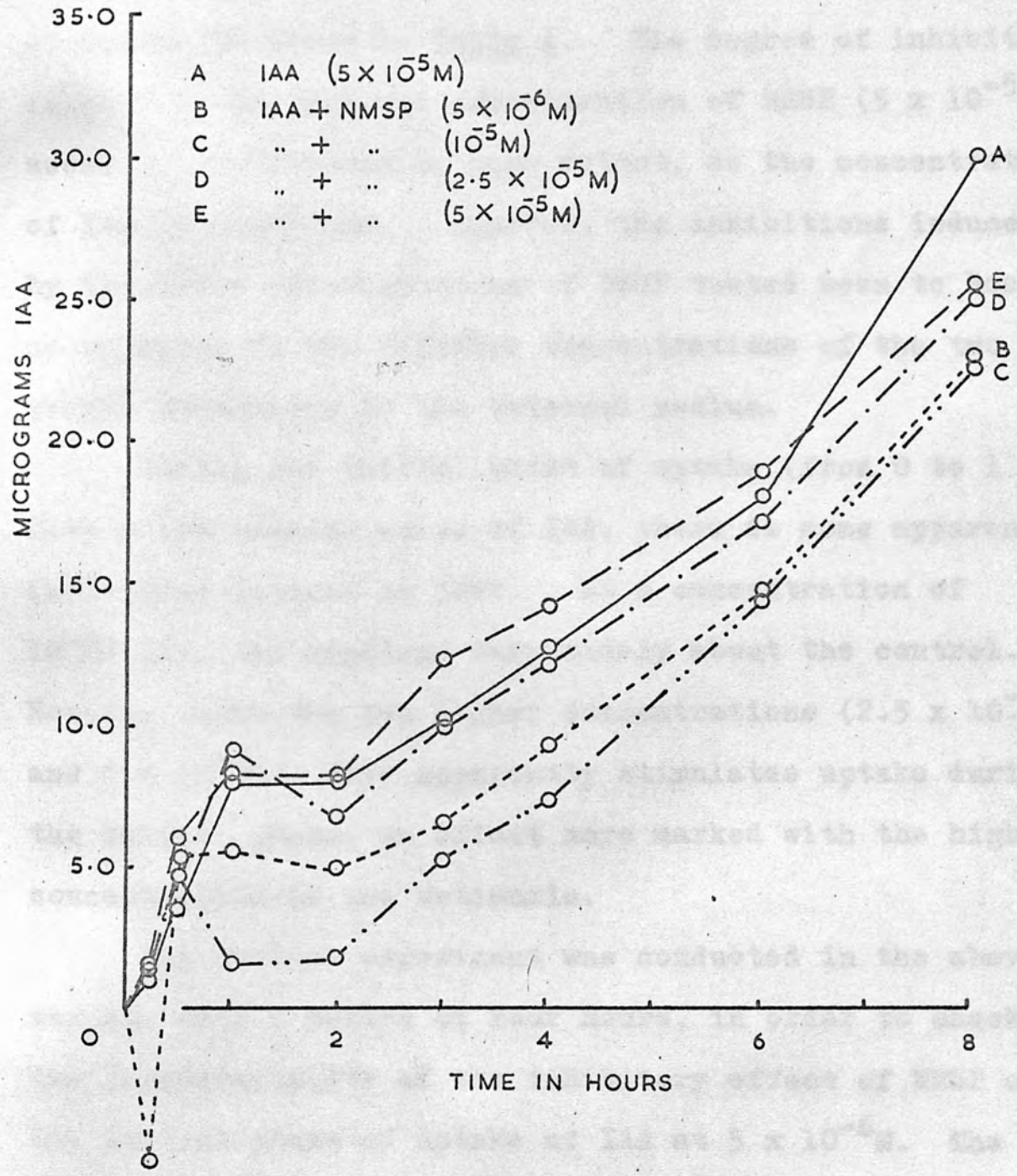

A IAA (2·5 × 10⁻⁵M)
B IAA + NMSP (5 × 10⁻⁶M)
C .. + .. (10⁻⁵M)
D .. + .. (2·5 × 10⁻⁵M)
E .. + .. (5 × 10⁻⁵M)
20·0
15·0
10·0
5·0
0
2
4
6
8
MICROGRAMS IAA
TIME IN HOURS
A
B
C
D
E

FIG. 13


35·0
30·0
25·0
20·0
15·0
10·0
5·0
0
MICROGRAMS IAA
A IAA (5 X 10⁻⁵M)
B IAA + NMSP (5 X 10⁻⁶M)
C .. + .. (10⁻⁵M)
D .. + .. (2·5 X 10⁻⁵M)
E .. + .. (5 X 10⁻⁵M)
2
4
6
8
TIME IN HOURS

is one of inhibition of IAA uptake by NMSP. Table 3 presents the data, derived from the above series of experiments, for the rate of IAA uptake as affected by NMSP. The percentage inhibition of the control rate of uptake is given in Table 4. The degree of inhibition induced by the highest concentration of NMSP (5×10^{-5}M) seems to be lessened to some extent, as the concentration of IAA is increased. However, the inhibitions induced by the other concentrations of NMSP tested seem to bear no relation to the relative concentrations of the two growth substances in the external medium.

During the initial phase of uptake (from 0 to 1 hour), with a low concentration of IAA, there is some apparent inhibition induced by NMSP. At a concentration of 10^{-5}M IAA, the readings vary widely about the control. However, with the two higher concentrations (2.5×10^{-5}M and 5×10^{-5}M), NMSP apparently stimulates uptake during the initial phase, an effect more marked with the higher concentration of the antiauxin.

One further experiment was conducted in the above series, over a period of four hours, in order to check the reproducibility of the inhibitory effect of NMSP on the initial phase of uptake of IAA at 5×10^{-6}M. The

TABLE 3

Expt. No.	IAA	NMSP 0	NMSP 5×10^{-6}M	NMSP 10^{-5}M	NMSP 2.5×10^{-5}M	NMSP 5×10^{-5}M
1	5×10^{-6}M	0.66	0.56	0.54	0.49	0.37
2	"	0.66	0.59	0.46	0.43	0.33
3	10^{-5}M	1.03	0.96	0.93	0.75	0.63
4	2.5×10^{-5}M	1.91	1.54	1.59	1.28	1.26
5	5×10^{-5}M	3.15	2.62	3.08	2.45	2.58

Effect of NMSP on the rate of IAA uptake by Avena coleoptile segments, expressed in micrograms IAA per hour.

TABLE 4

Expt. No.	IAA	NMSP			
		5×10^{-6}M	10^{-5}M	2.5×10^{-5}M	5×10^{-5}M
1	5×10^{-6}M	14.4	18.4	24.6	44.0
2	"	10.6	30.3	35.2	49.8
3	10^{-5}M	7.2	9.7	27.7	38.9
4	2.5×10^{-5}M	19.0	16.8	32.8	34.0
5	5×10^{-5}M	17.0	2.3	22.4	18.1

Percentage inhibition of the rate of IAA uptake induced by NMSP.

effect of NMSP at 10^{-5}M was examined, and the experiment more frequently sampled during the first hour. As may be seen from the fluorescence readings presented in Table 5 there is no detectable loss in fluorescence intensity over one hour, and in contrast to the results obtained in Experiments 1 and 2 of this series, there is no significant effect of NMSP at this concentration on the uptake of IAA at 5×10^{-6}M over 4 hours.

As the pattern of interaction between IAA and NSMP shown by the preceding experiments was felt to be unsatisfactory, a similar set of experiments, using the same growth substances, was carried out under identical conditions early in 1964. The results are described below:

Experiment 1.
- A. IAA (10^{-5}M) Control
- B. IAA (") + NMSP (10^{-5}M)
- C. IAA (") + NMSP (2.5×10^{-5}M)
- D. IAA (") + NMSP (5×10^{-5}M)

Data: Fig.14 (Table XII)

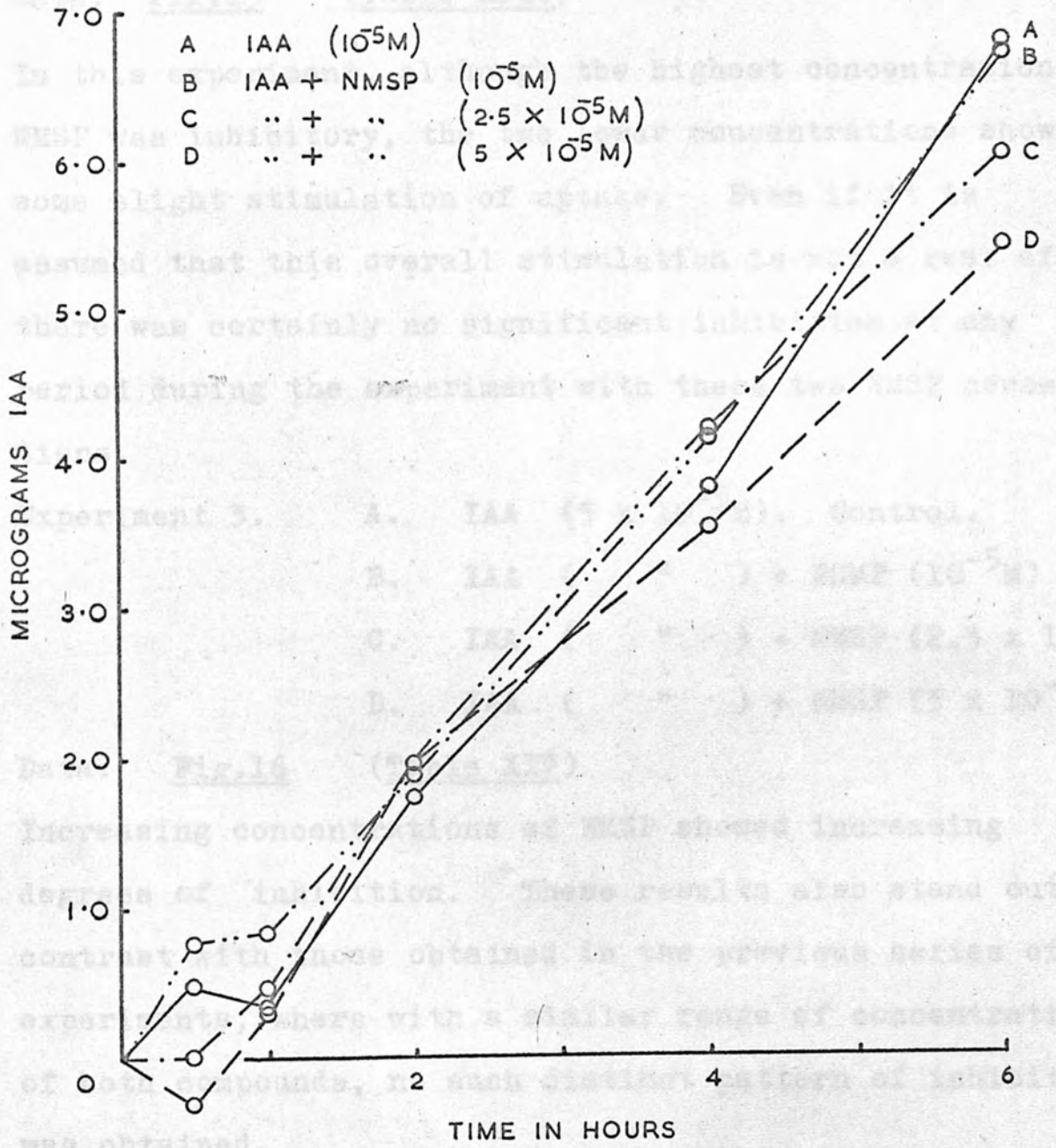
Uptake was measured over a period of 6 hours. Again some inhibition of uptake was recorded with these concentrations of NMSP, but the degree was much less than obtained with the earlier experiments.

TABLE 5

GROWTH SUBST.		FLUORESCENCE INTENSITY							
IAA	NMSP	In.	5 m	10 m	15 m	30 m	1 hr	2 hr	4 hr
5×10^{-6}M	____	28.1	28.0	28.1	26.5	28.0	28.0	27.0	22.9
5×10^{-6}M	10^{-5}M	28.1	28.9	28.5	27.1	27.5	27.8	26.5	23.0

Batches of 100 Avena coleoptile segments were floated on solutions of IAA (5×10^{-6}M) and IAA + NMSP (10^{-5}M). The media were sampled at frequent intervals and the fluorescence intensity measured. In the Table, intensity is expressed in arbitrary units of the photometer scale.

FIG. 14


A IAA (10^{-5}M)
B IAA + NMSP (10^{-5}M)
C " + " (2.5×10^{-5}M)
D " + " (5×10^{-5}M)
MICROGRAMS IAA
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0
2
4
6
TIME IN HOURS
A
B
C
D

Experiment 2. A. IAA (2.5×10^{-5}M) Control.

B. IAA (") +NMSP (10^{-5}M)

C. IAA (") +NMSP (2.5×10^{-5}M)

D. IAA (") +NSMP (5×10^{-5}M)

Data: Fig.15 (Table XIII)

In this experiment, although the highest concentration of NMSP was inhibitory, the two lower concentrations showed some slight stimulation of uptake. Even if it is assumed that this overall stimulation is not a real effect, there was certainly no significant inhibition at any period during the experiment with these two NMSP concentrations.

Experiment 3. A. IAA (5×10^{-5}M). Control.

B. IAA (") + NSMP (10^{-5}M)

C. IAA (") + NMSP (2.5×10^{-5}M)

D. IAA (") + NMSP (5×10^{-5}M).

Data: Fig.16 (Table XIV)

Increasing concentrations of NMSP showed increasing degrees of inhibition. These results also stand out in contrast with those obtained in the previous series of experiments, where with a similar range of concentrations of both compounds, no such distinct pattern of inhibition was obtained.

FIG. 15

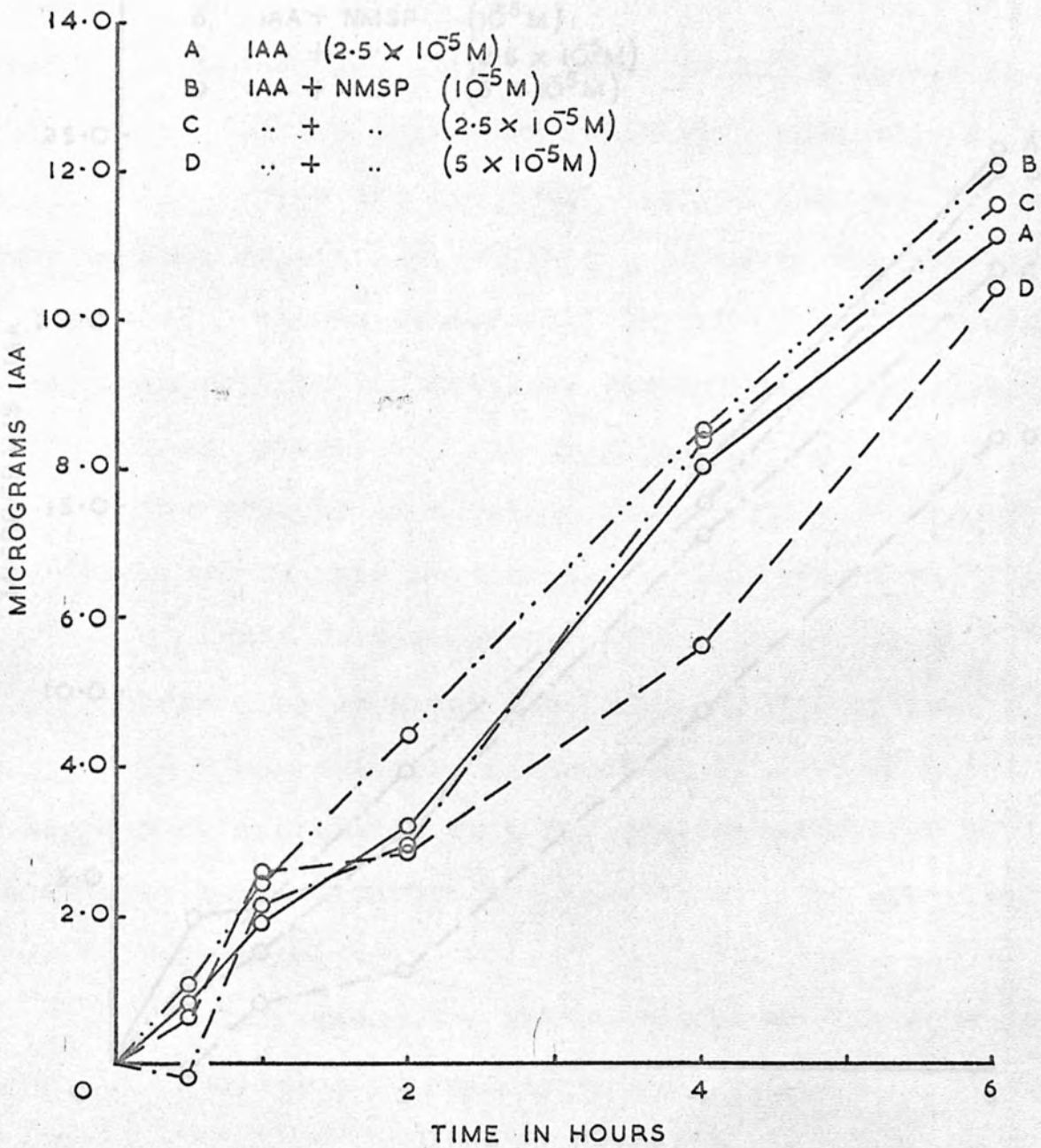
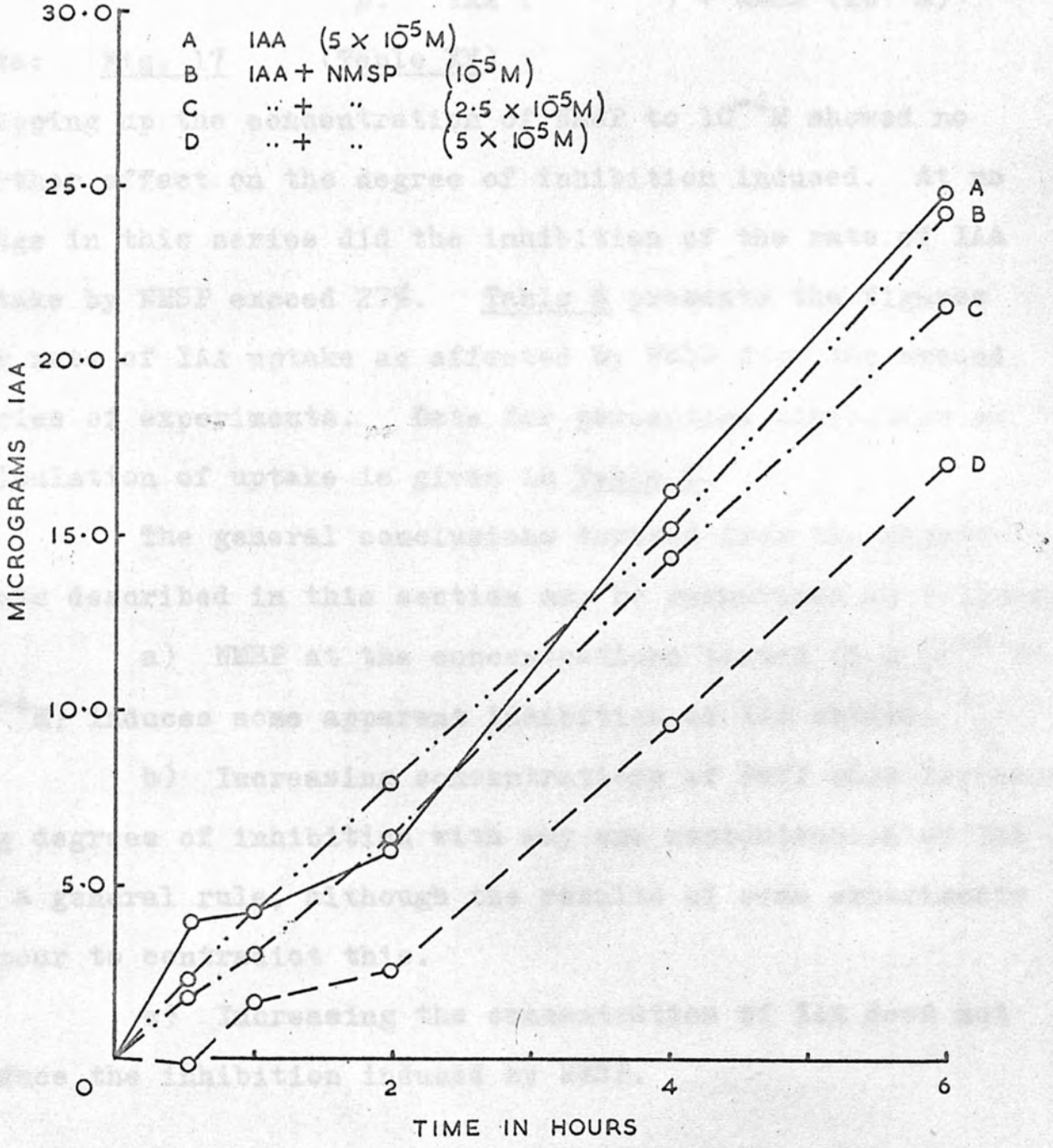

A IAA (2·5 × 10⁻⁵ M)
B IAA + NMSP (10⁻⁵ M)
C .. + .. (2·5 × 10⁻⁵ M)
D .. + .. (5 × 10⁻⁵ M)
MICROGRAMS IAA
14·0
12·0
10·0
8·0
6·0
4·0
2·0
0
2
4
6
TIME IN HOURS
B
C
A
D

FIG. 16


A IAA (5 × 10⁻⁵M)
B IAA + NMSP (10⁻⁵M)
C ·· + ·· (2·5 × 10⁻⁵M)
D ·· + ·· (5 × 10⁻⁵M)
30·0
25·0
20·0
15·0
10·0
5·0
0
2
4
6
MICROGRAMS IAA
TIME IN HOURS
A
B
C
D

Experiment 4. A. IAA (5×10^{-5}M) Control.

B. IAA (") + NMSP (2.5×10^{-5}M)

C. IAA (") + NMSP (5×10^{-5}M)

D. IAA (") + NMSP (10^{-4}M)

Data: Fig. 17 (Table XV)

Stepping up the concentration of NMSP to 10^{-4}M showed no further effect on the degree of inhibition induced. At no stage in this series did the inhibition of the rate of IAA uptake by NMSP exceed 27%. Table 6 presents the figures for rate of IAA uptake as affected by NMSP from the second series of experiments. Data for percentage inhibition or stimulation of uptake is given in Table 7.

The general conclusions derived from the experiments described in this section may be summarized as follows:

a) NMSP at the concentrations tested (5×10^{-6} to 10^{-4}M) induces some apparent inhibition of IAA uptake.

b) Increasing concentrations of NMSP show increasing degrees of inhibition with any one concentration of IAA as a general rule, although the results of some experiments appear to contradict this.

c) Increasing the concentration of IAA does not reduce the inhibition induced by NMSP.

FIG. 17

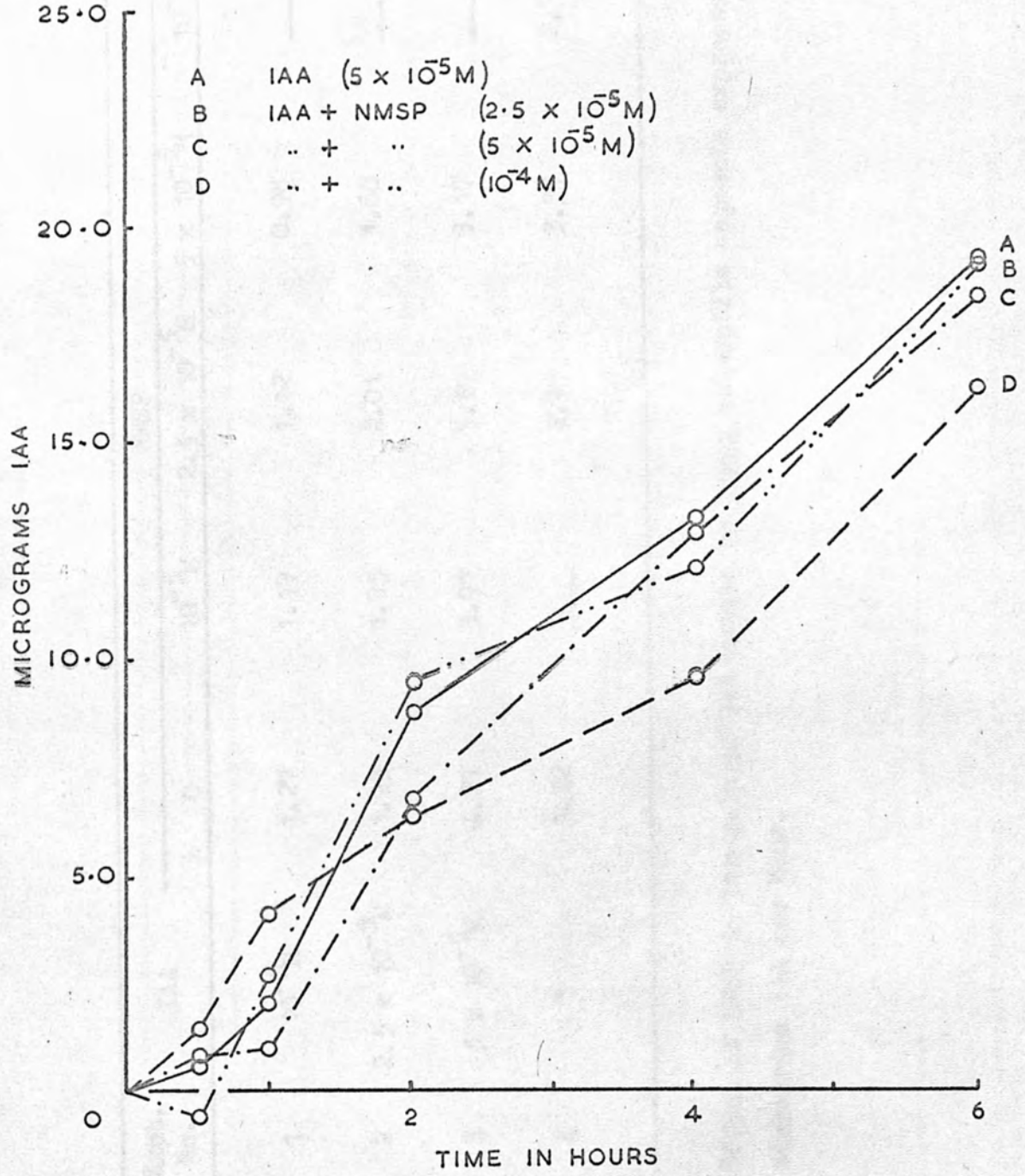

A IAA (5 × 10⁻⁵M)
B IAA + NMSP (2·5 × 10⁻⁵M)
C .. + .. (5 × 10⁻⁵M)
D .. + .. (10⁻⁴M)
25·0
20·0
15·0
10·0
5·0
0
MICROGRAMS IAA
2
4
6
TIME IN HOURS
A
B
C
D

TABLE 6

Expt. No.	IAA	NMSP				
		0	10^{-5}M	2.5 x 10^{-5}M	5 x 10^{-5}M	10^{-4}M
1	10^{-5}M	1.27	1.19	1.12	0.98	____
2	2.5 x 10^{-5}M	1.93	1.95	2.01	1.60	____
3	5 x 10^{-5}M	4.27	3.97	3.59	3.17	____
4	"	3.22	____	2.95	3.39	2.38

Effect of NMSP on the rate of IAA uptake by Avena coleoptile segments expressed in micrograms IAA per hour.

TABLE 7

Expt. No.	IAA	NMSP			
		10^{-5}M	2.5 x 10^{-5}M	5 x 10^{-5}M	10^{-4}M
1	10^{-5}M	-6.5	-11.7	-22.6	____
2	2.5 x 10^{-5}M	+1.1	+ 4.2	-17.2	____
3	5 x 10^{-5}M	-7.2	-16.0	-25.9	____
4	"	____	- 8.4	+ 5.0	-26.3

Percentage inhibition (-) or stimulation (+) of the rate of IAA uptake induced by NMSP.

IV

MATERIAL: *Avena* coleoptile

METHOD: Fluorescence assay

GROWTH-SUBSTANCES: IAA + 2,4-D

In the present work, only one preliminary experiment was carried out to examine the effect of 2,4-D on the penetration of IAA into segments of *Avena* coleoptile. Further work on the uptake interactions between IAA and 2,4-D was conducted employing maize mesocotyl tissue and the results are reported in a later section. 2,4-D itself is non-fluorescing and does not interfere with the fluorescence of IAA at concentrations tested in the present investigations.

Experiment:

A. IAA (10^{-5}M) Control

B. IAA (") + 2,4-D (10^{-5}M)

C. IAA (") + 2,4-D (2.5×10^{-5}M)

D. IAA (") + 2,4-D (5×10^{-5}M)

Uptake was measured over a period of 6 hours. Measurements taken of the fluorescence intensity of test samples during the course of the experiment, expressed in arbitrary units of the photometer scale, are presented in Table 8. Uptake values calculated for the control lot and expressed in micrograms IAA per 100 segments are also included in the Table.

It will be seen from the results that no inhibition

TABLE 8

AUXINS		FLUORESCENCE READINGS						IAA UPTAKE				
IAA	2,4-D	In,(b)	30 m	1 hr	2 hr	4 hr	6 hr	30 m	1 hr	2 hr	4 hr	6 hr
10^{-5}M	_____	48.0	47.0	45.5	43.0	40.0	31.0	0.33	0.79	1.52	2.48	4.65
"	10^{-5}M	49.0	48.9	48.0	42.0	38.0	30.5					
"	2.5 x 10^{-5}M	47.5	46.5	46.0	43.0	36.0	30.0					
"	5 x 10^{-5}M	47.0	47.9	45.0	42.0	36.5	30.0					

Effect of 2,4-D on IAA uptake by Avena coleoptile segments. Fluorescence intensity of test samples taken during the course of the experiment is expressed in arbitrary units of the photometer scale. Uptake values of the IAA control are expressed in micrograms IAA per 100 segments.

of IAA uptake by 2,4-D is evidenced at any concentration tested.

V

MATERIAL: Avena coleoptile

METHOD: Fluorescence assay

GROWTH-SUBSTANCE: NAA

NAA is a strongly fluorescing auxin and remains stable during activation. Three experiments, described below, were conducted to estimate NAA uptake by Avena coleoptile segments from a series of concentrations ranging from 5×10^{-6}M to 5×10^{-5}M. Uptake was measured over periods extending from 4 to 6 hours.

Experiment 1. A. NAA (5×10^{-6}M)

B. NAA (10^{-5}M)

C. NAA (2.5×10^{-5}M)

D. NAA (5×10^{-5}M)

Data: Fig.18 (Table XVI)

Experiment 2. A. NAA (5×10^{-6}M)

B. NAA (10^{-5}M)

C. NAA (2.5×10^{-5}M)

D. NAA (5×10^{-5}M)

Data: Fig.19 (Table XVII)

Experiment 3. A. NAA (10^{-5}M)

B. NAA (2.5×10^{-5}M)

C. NAA (5×10^{-5}M)

Data: Fig.20 (Table XVIII)

FIG. 18: UPTAKE OF NAA

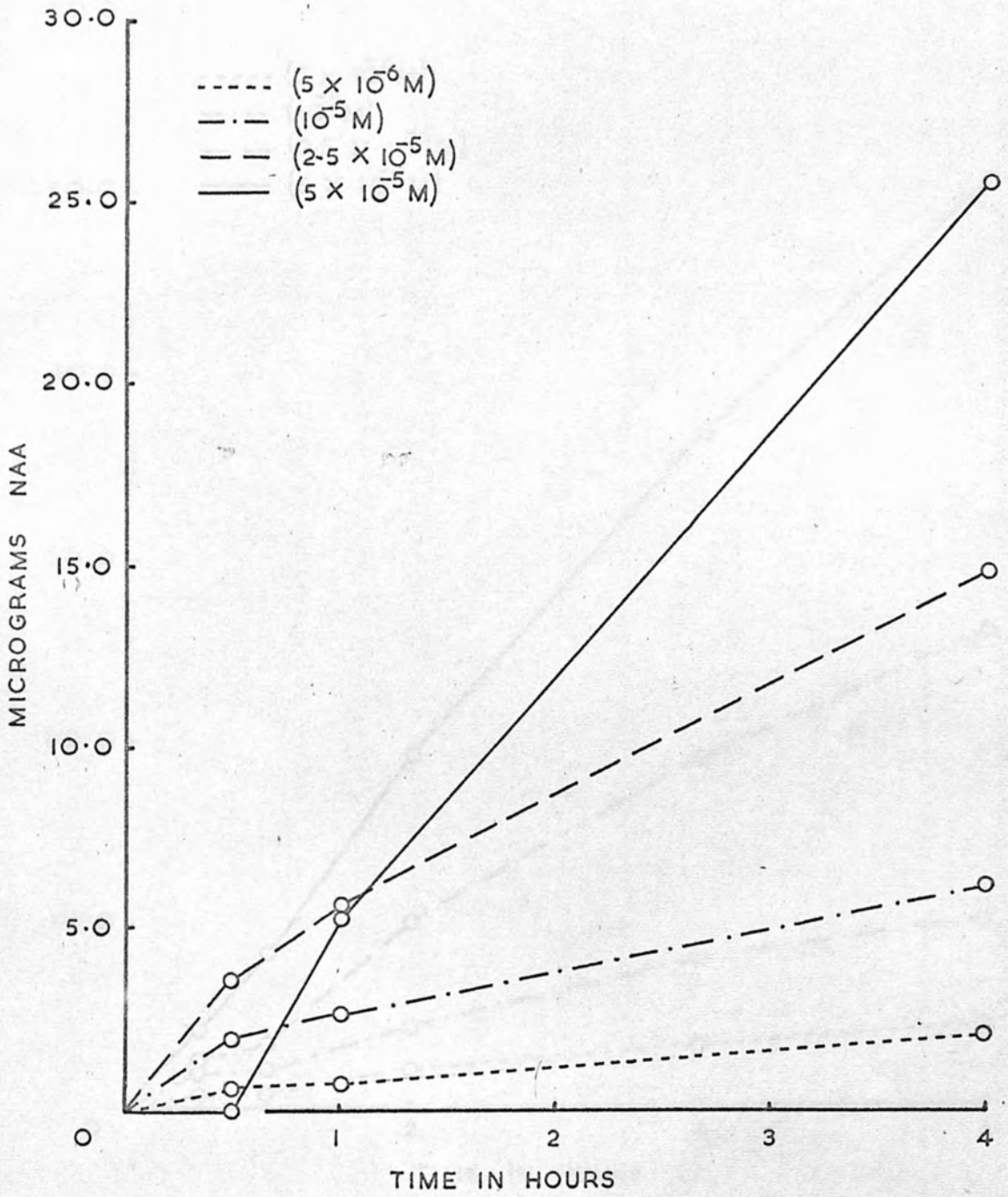


FIG. 19: UPTAKE OF NAA

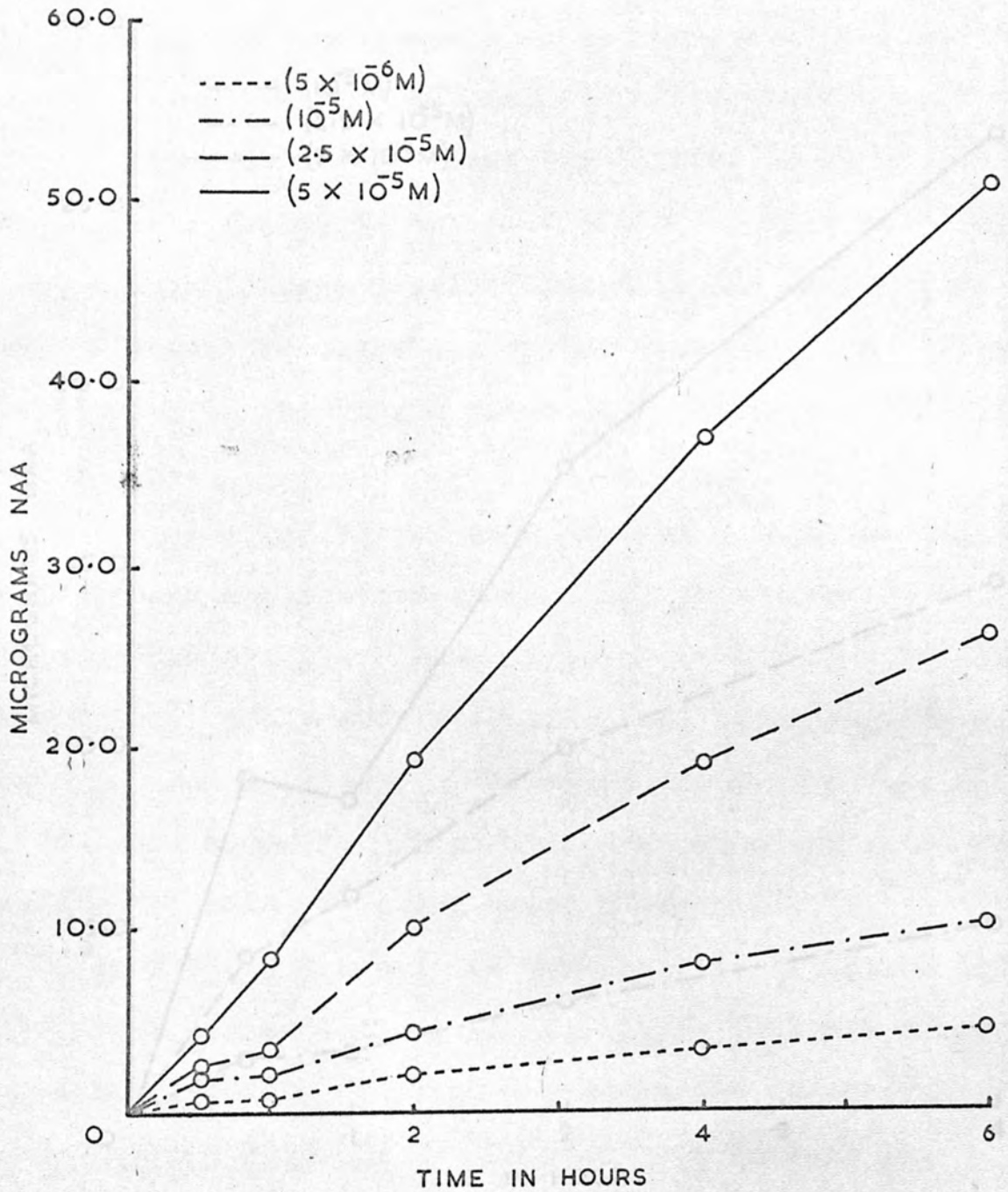
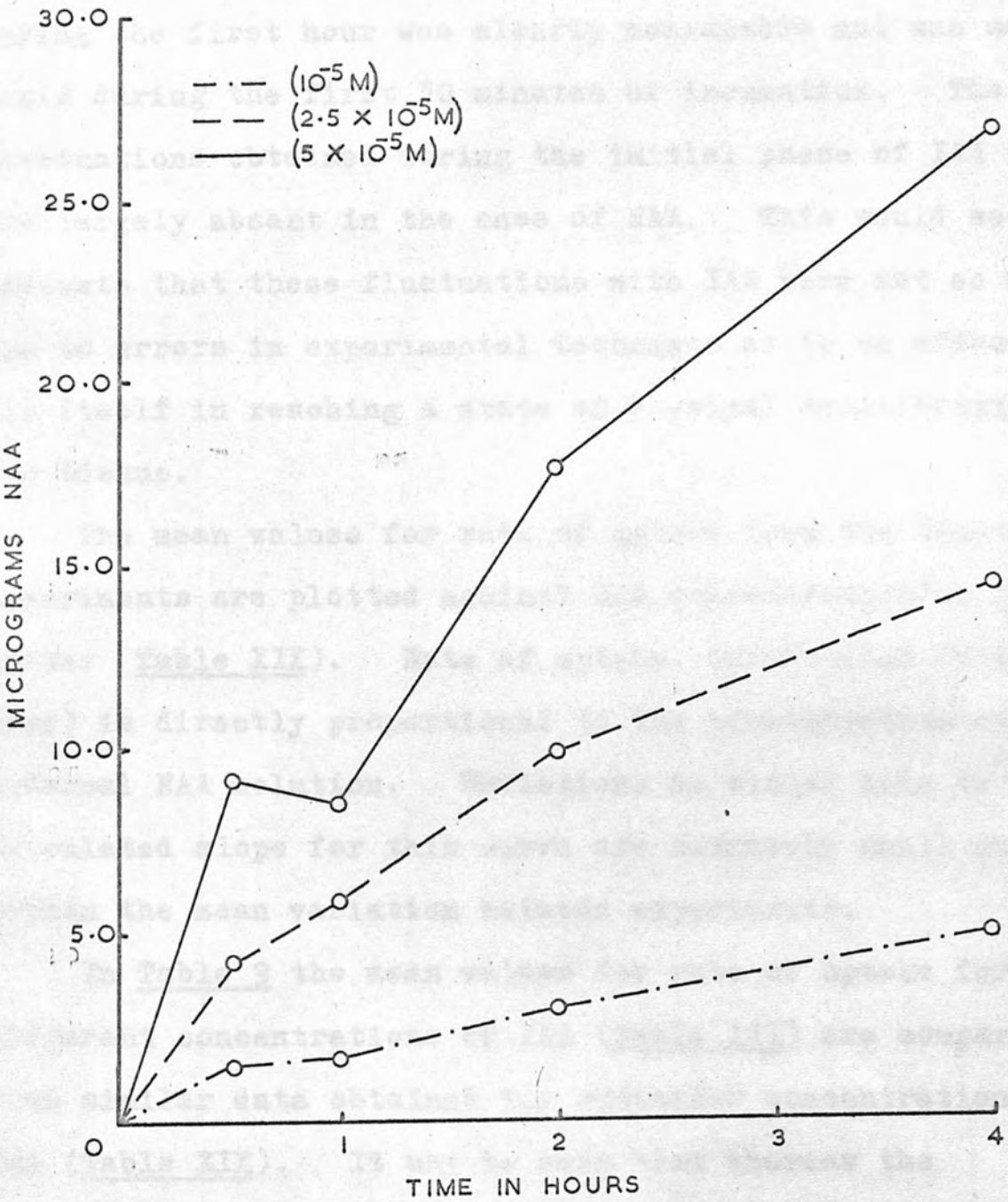


FIG. 20: UPTAKE OF NAA



When uptake was plotted against time (Figs. 18-20), as a general rule, smooth uptake curves were obtained. Over 6 hours there was little fall in the rate of uptake. Uptake during the first hour was clearly measurable and was most rapid during the first 30 minutes of incubation. The fluctuations obtained during the initial phase of IAA uptake are largely absent in the case of NAA. This would seem to indicate that these fluctuations with IAA were not so much due to errors in experimental technique as to an effect of IAA itself in reaching a state of physical equilibrium with the tissue.

The mean values for rate of uptake from the three experiments are plotted against NAA concentration in Fig.21 (Data: Table XIX). Rate of uptake, (calculated from 0 hour) is directly proportional to the concentration of the external NAA solution. Deviations on either side of the calculated slope for this curve are extremely small and well within the mean variation between experiments.

In Table 9 the mean values for rate of uptake for different concentrations of IAA (Table III) are compared with similar data obtained for equimolar concentrations of NAA (Table XIX). It may be seen that whereas the

FIG. 21

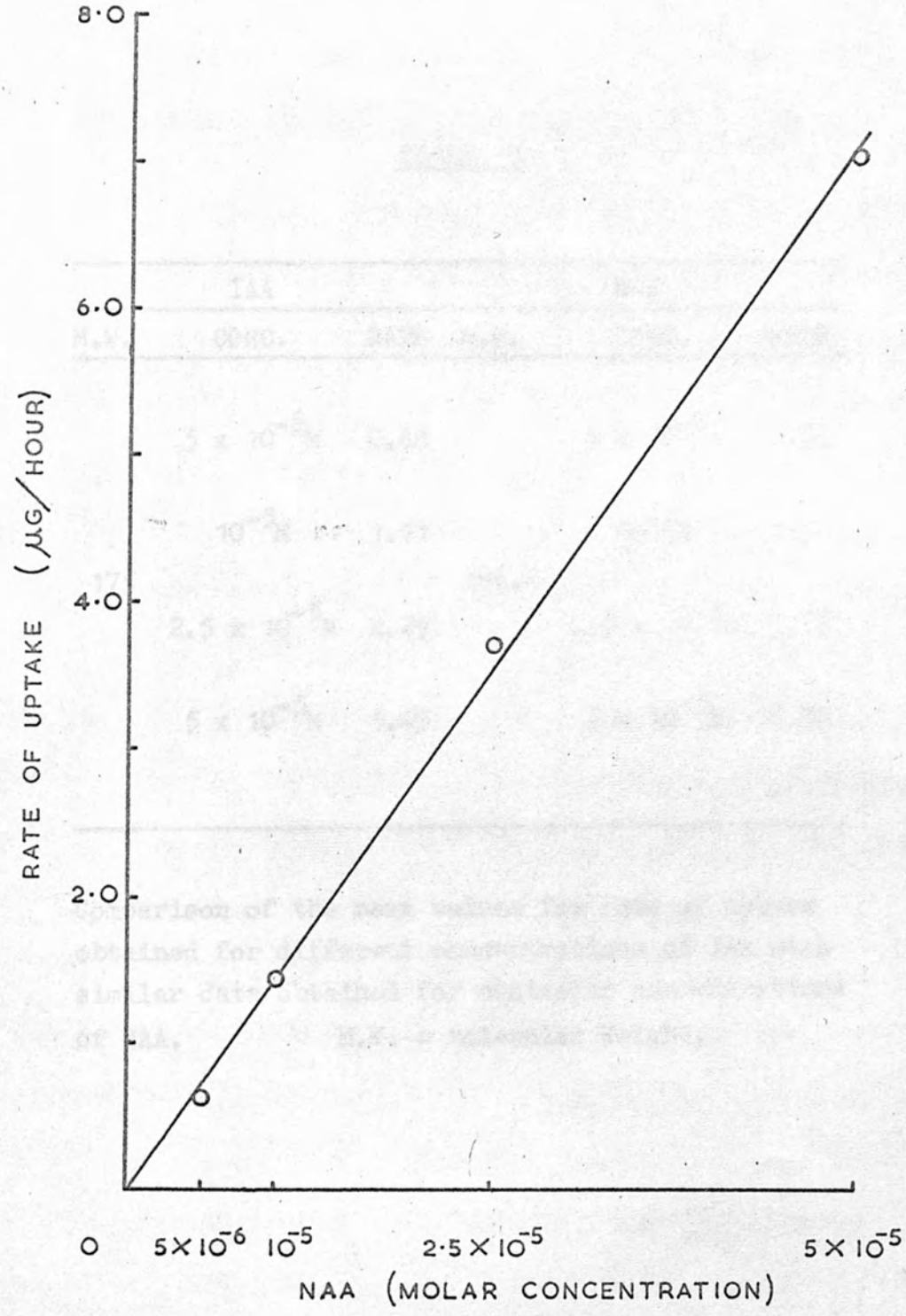

8·0
6·0
4·0
2·0
RATE OF UPTAKE (µG/HOUR)
0
5×10⁻⁶
10⁻⁵
2·5×10⁻⁵
5×10⁻⁵
NAA (MOLAR CONCENTRATION)

TABLE 9

IAA			NAA		
M.W.	CONC.	RATE	M.W.	CONC.	RATE
	5×10^{-6}M	0.68		5×10^{-6}M	0.64
	10^{-5}M	1.11		10^{-5}M	1.42
175			186.2		
	2.5×10^{-5}M	2.29		2.5×10^{-5}M	3.72
	5×10^{-5}M	5.03		5×10^{-5}M	7.05

Comparison of the mean values for rate of uptake obtained for different concentrations of IAA with similar data obtained for equimolar concentrations of NAA. M.W. = Molecular Weight.

difference in the molecular weights of the two compounds is very small (6%), the difference in the rate of uptake as a function of concentration is approximately 30%, NAA being accumulated in the tissue faster than IAA.

VI

MATERIAL: Avena coleoptile

METHOD: Fluorescence assay

GROWTH-SUBSTANCES: NAA + 2,4-D

NAA + NMSP

A range of concentrations from 10^{-5}M to 5 x 10^{-5}M of 2,4-D and NMSP were tested for their effects on the uptake of NAA. The experiments were conducted so that both compounds were tested in various concentrations on a single NAA concentration in each experiment, in order to compare their respective effects more rigidly by reducing inter-experimental variations. The experiments are described below:

Experiment 1. A. NAA (10^{-5}M) Control

B. NAA (") + 2,4-D (10^{-5}M)

C. NAA (") + 2,4-D (2.5 x 10^{-5}M)

D. NAA (") + NMSP (10^{-5}M)

Data: Fig.22 (Table XXI)

The results show no effect by either of the two compounds at the above concentrations.

Experiment 2. A. NAA (10^{-5}M) Control

B. NAA (") + 2,4-D (5 x 10^{-5}M)

C. NAA (") + NMSP (2.5 x 10^{-5}M)

D. NAA (") + NMSP (5 x 10^{-5}M)

Data: Fig.23 (Table XXII)

FIG. 22

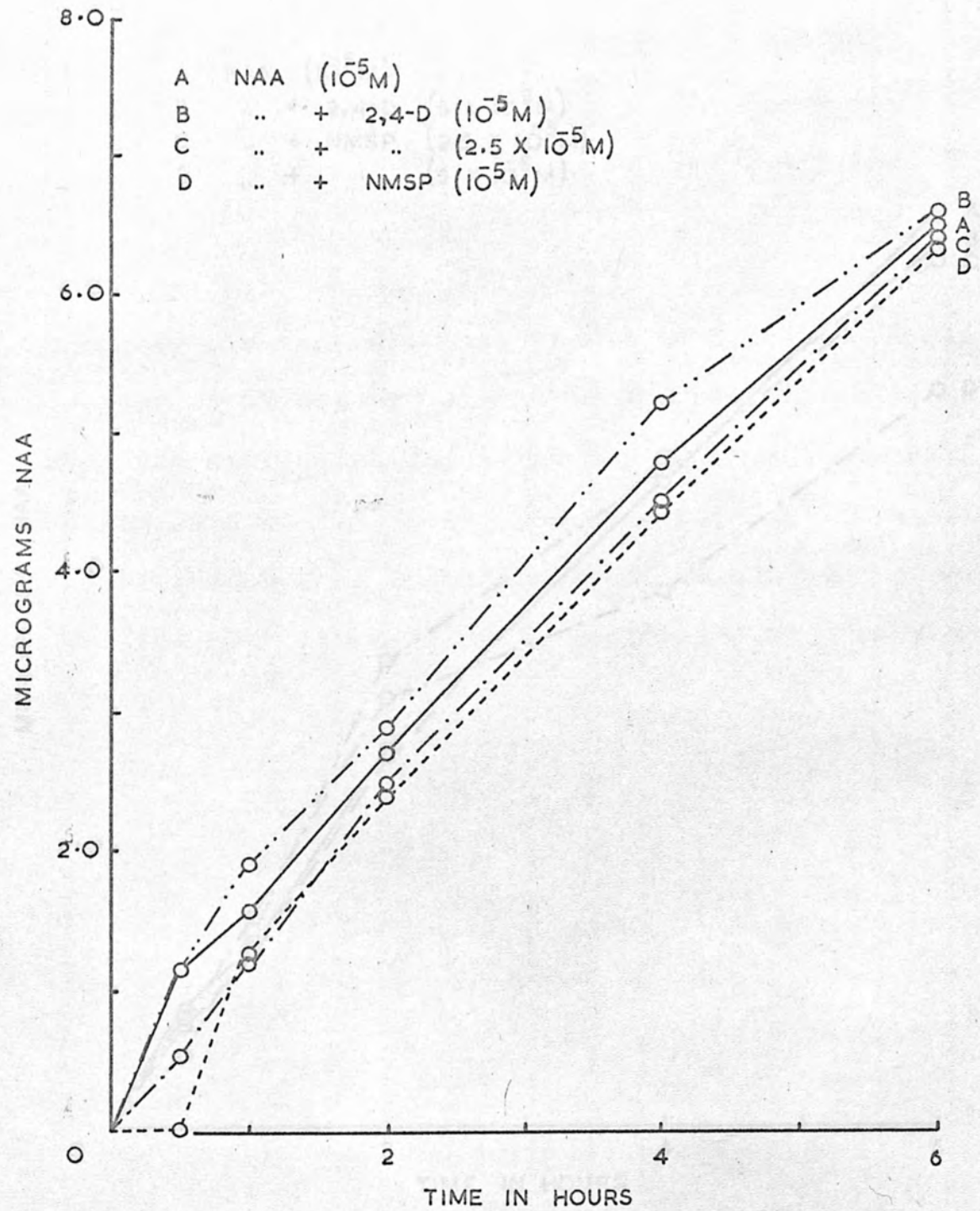
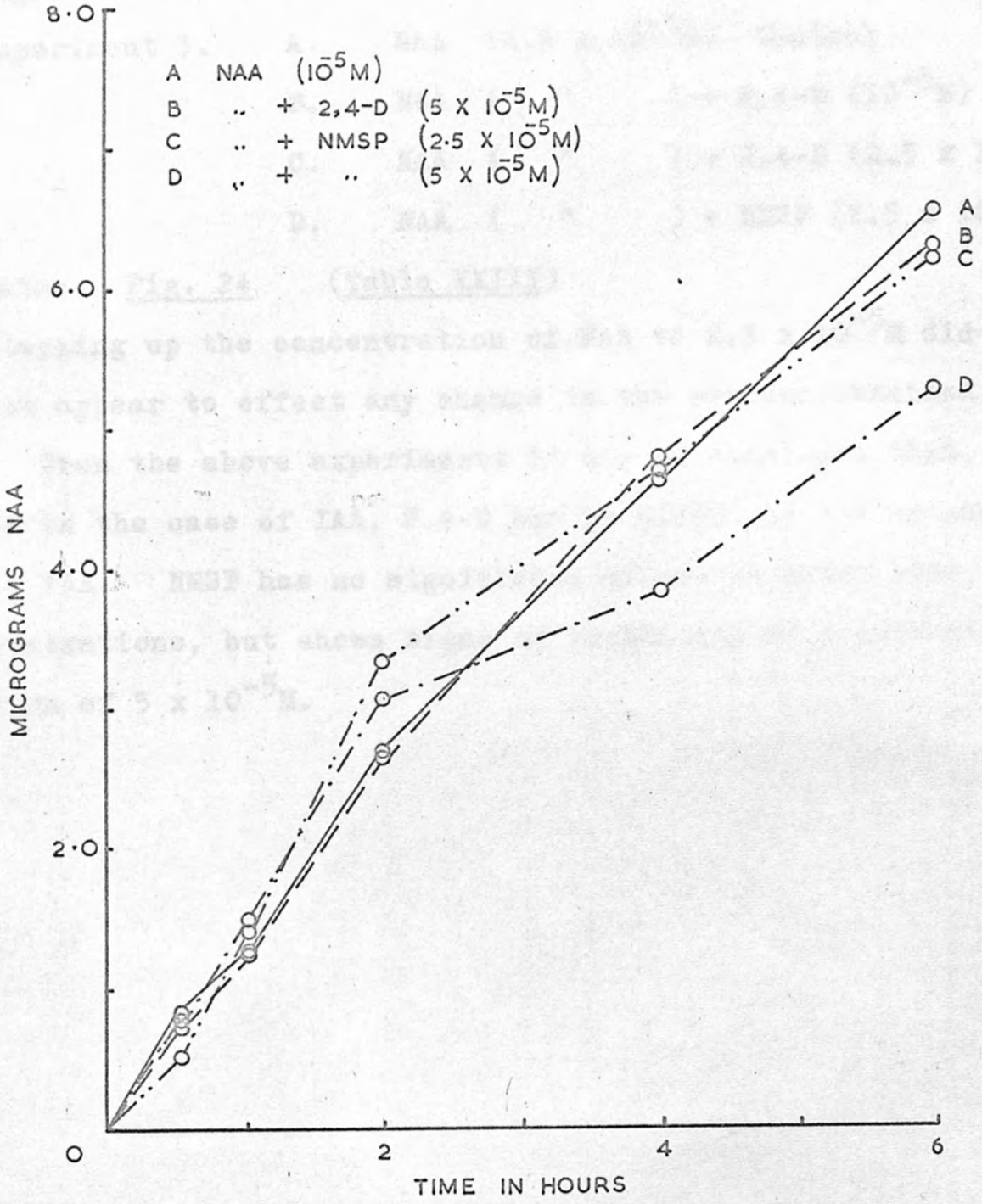

8·0
6·0
4·0
2·0
0
2
4
6
MICROGRAMS NAA
TIME IN HOURS
A NAA (10^{-5}M)
B .. + 2,4-D (10^{-5}M)
C .. + .. (2.5 X 10^{-5}M)
D .. + NMSP (10^{-5}M)
B
A
C
D

FIG. 23


A NAA (10^{-5}M)
B .. + 2,4-D (5 X 10^{-5}M)
C .. + NMSP (2.5 X 10^{-5}M)
D .. + .. (5 X 10^{-5}M)
8·0
6·0
4·0
2·0
0
2
4
6
A
B
C
D
MICROGRAMS NAA
TIME IN HOURS

A fairly significant inhibition was evidenced only by the highest concentration of NMSP (5×10^{-5}M).

Experiment 3. A. NAA (2.5×10^{-5}M) Control

B. NAA (") + 2,4-D (10^{-5}M)

C. NAA (") + 2,4-D (2.5×10^{-5}M)

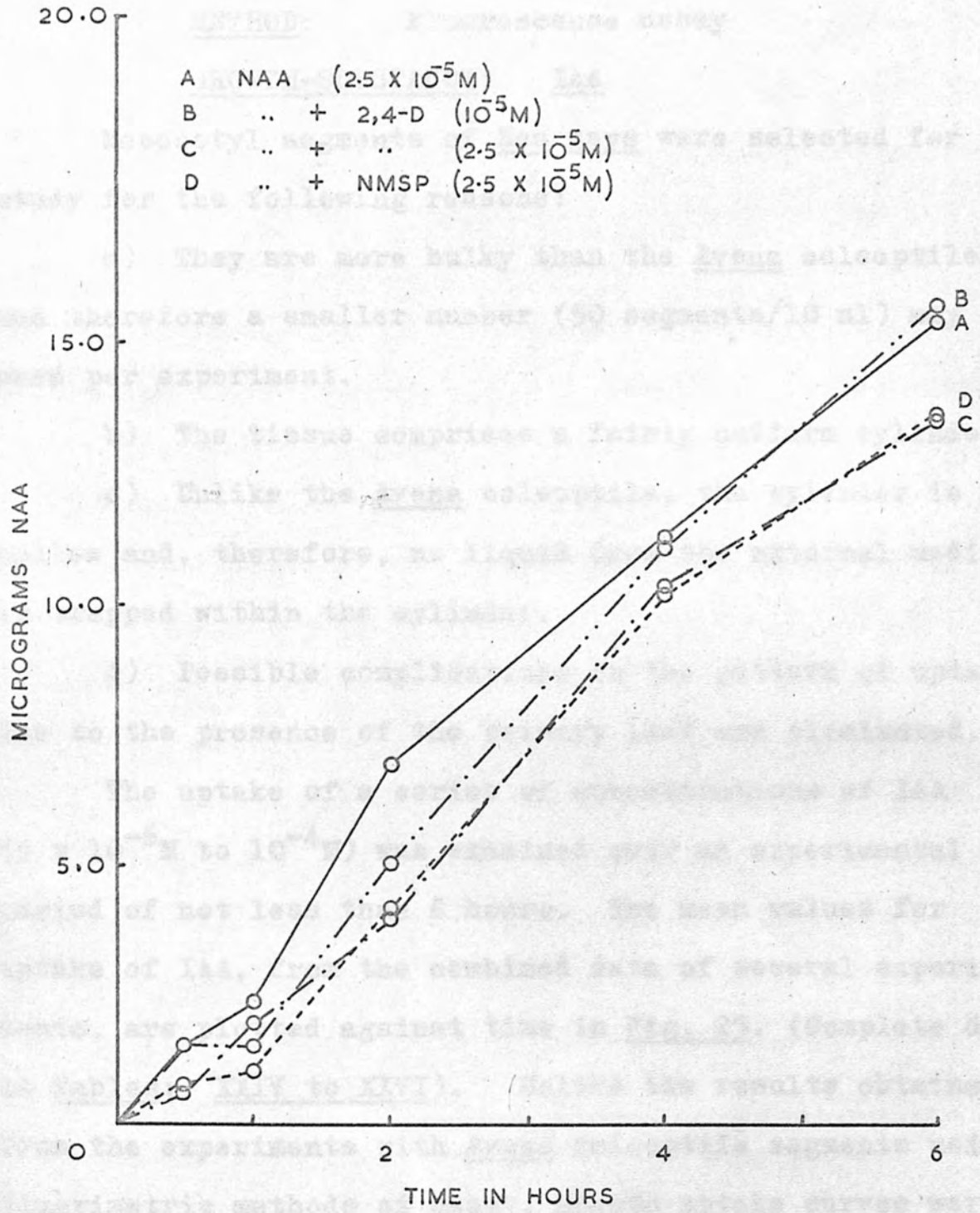
D. NAA (") + NMSP (2.5×10^{-5}M)

Data: Fig. 24 (Table XXIII)

Stepping up the concentration of NAA to 2.5×10^{-5}M did not appear to effect any change in the pattern obtained.

From the above experiments it may be concluded that, as in the case of IAA, 2,4-D has no effect on the uptake of NAA. NMSP has no significant effect at lower concentrations, but shows signs of inhibition at a concentration of 5×10^{-5}M.

FIG. 24


20·0
15·0
10·0
5·0
0
2
4
6
MICROGRAMS NAA
TIME IN HOURS
A NAA (2·5 X 10⁻⁵M)
B .. + 2,4-D (10⁻⁵M)
C .. + .. (2·5 X 10⁻⁵M)
D .. + NMSP (2·5 X 10⁻⁵M)
B
A
D
C

VII (a)

MATERIAL: Zea mays - mesocotyl

METHOD: Fluorescence assay

GROWTH-SUBSTANCE: IAA

Mesocotyl segments of Zea mays were selected for study for the following reasons:

a) They are more bulky than the Avena coleoptiles and therefore a smaller number (50 segments/10 ml) may be used per experiment.

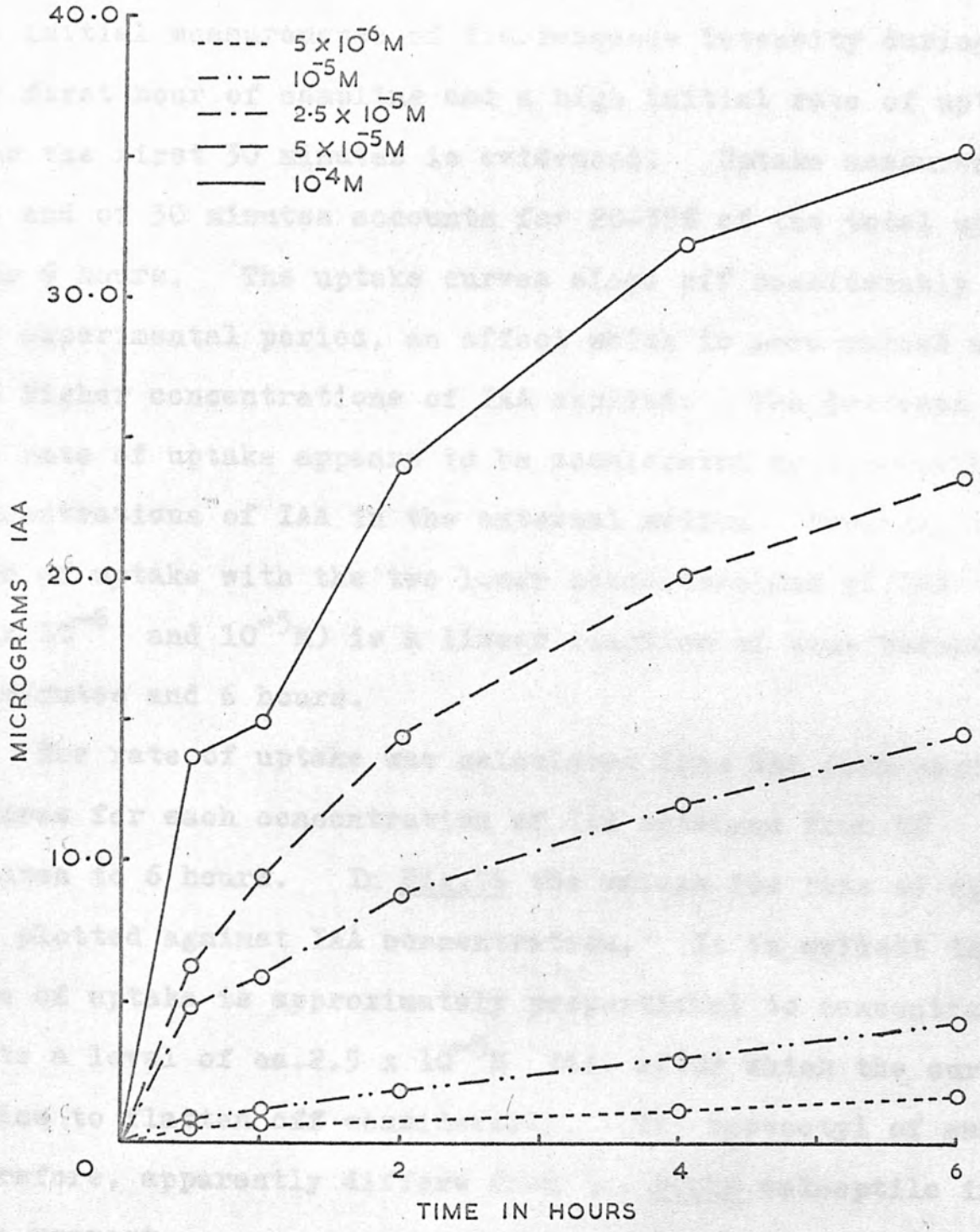
b) The tissue comprises a fairly uniform cylinder.

c) Unlike the Avena coleoptile, the cylinder is not hollow and, therefore, no liquid from the external medium is trapped within the cylinder.

d) Possible complications in the pattern of uptake due to the presence of the primary leaf are eliminated.

The uptake of a series of concentrations of IAA (5×10^{-6}M to 10^{-4}M) was examined over an experimental period of not less than 6 hours. The mean values for uptake of IAA, from the combined data of several experiments, are plotted against time in Fig. 25. (Complete data in Tables: XXIV to XXVI). Unlike the results obtained from the experiments with Avena coleoptile segments using fluorimetric methods of assay, smooth uptake curves were obtained with this material. The mean variation between

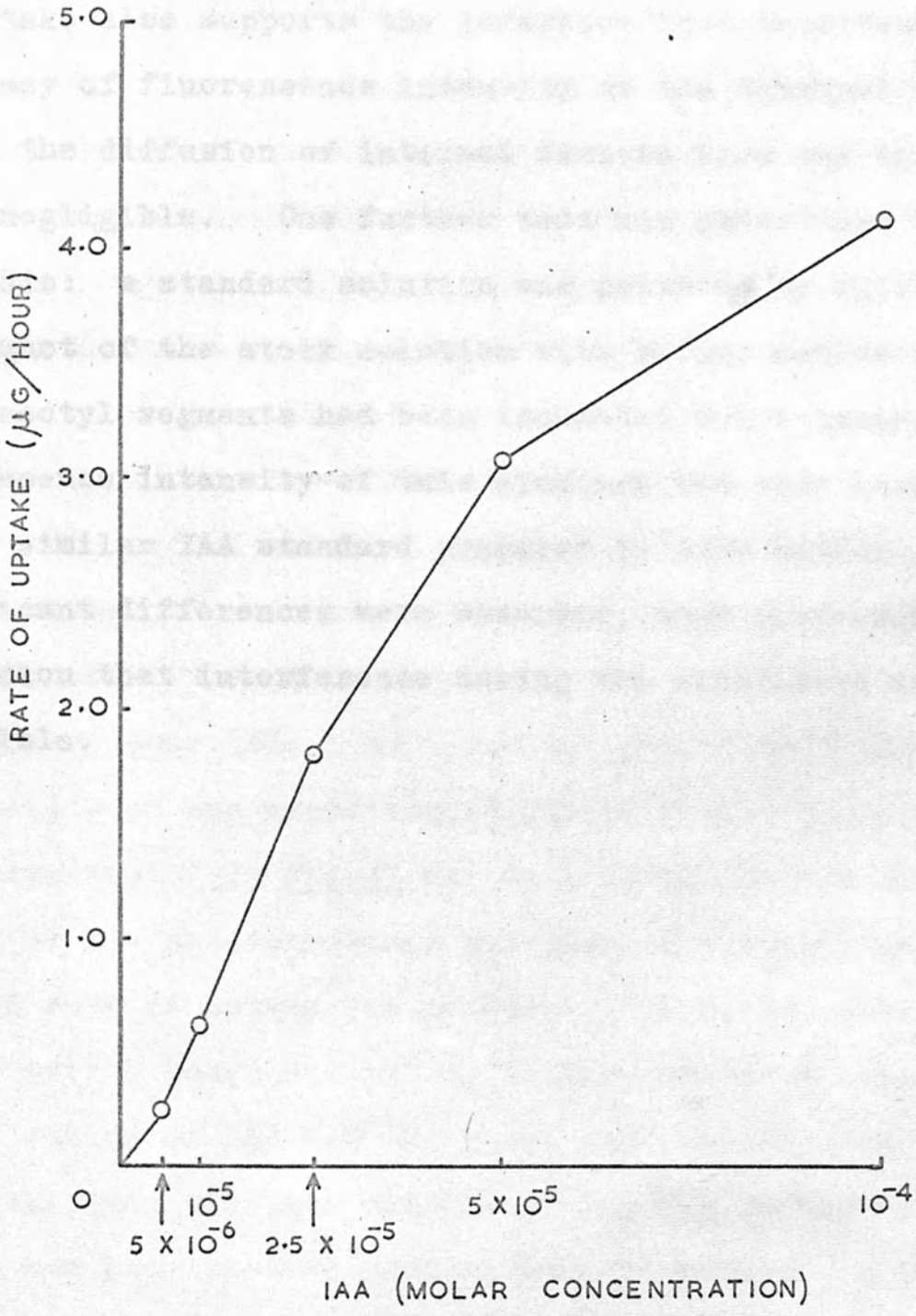
FIG. 25: UPTAKE OF IAA



experiments is small, and the uptake figures appear to be very reproducible. Very little fluctuation was observed in the initial measurements of fluorescence intensity during the first hour of sampling and a high initial rate of uptake over the first 30 minutes is evidenced. Uptake measured at the end of 30 minutes accounts for 20-35% of the total uptake over 6 hours. The uptake curves slope off considerably over the experimental period, an effect which is more marked with the higher concentrations of IAA applied. The decrease in the rate of uptake appears to be accelerated by increasing concentrations of IAA in the external medium. However, the rate of uptake with the two lower concentrations of IAA (5×10^{-6} and 10^{-5}M) is a linear function of time between 30 minutes and 6 hours.

The rate of uptake was calculated from the mean uptake figures for each concentration of IAA obtained from 30 minutes to 6 hours. In Fig.26 the values for rate of uptake are plotted against IAA concentration. It is evident that rate of uptake is approximately proportional to concentration up to a level of ca.2.5×10^{-5}M IAA, after which the curve begins to flatten off considerably. The mesocotyl of maize, therefore, apparently differs from the Avena coleoptile in this respect.

FIG. 26


5·0
4·0
3·0
2·0
1·0
0
RATE OF UPTAKE (µG/HOUR)
5 X 10⁻⁶
10⁻⁵
2·5 X 10⁻⁵
5 X 10⁻⁵
10⁻⁴
IAA (MOLAR CONCENTRATION)

When mesocotyl segments were incubated in buffer medium alone, there was no indication of the diffusion of any fluorescing compounds out of the tissue. The pattern of IAA uptake also supports the inference that interference in the assay of fluorescence intensity of the external medium, due to the diffusion of internal factors from the tissue is quite negligible. One further test was undertaken to confirm this: a standard solution was prepared by diluting an aliquot of the stock solution with buffer medium in which 50 mesocotyl segments had been incubated for 4 hours. The fluorescence intensity of this standard was then compared with a similar IAA standard prepared in pure buffer. No significant differences were observed, thus confirming the concludion that interference during the experiment was negligible.

VII (b)

MATERIAL: Zea mays - mesocotyl

METHOD: Radioactivity assay

GROWTH-SUBSTANCE: IAA - C^{14}

Data for the uptake of labelled IAA - C^{14} by segments of maize mesocotyl have been compiled from a series of 19 experiments in which the uptake of various concentrations of IAA* was assayed. In Table 10 these data have been tabulated so as to indicate the mean deviation between experiments. The reproducibility of the figures for uptake at any sampling period and for any concentration of IAA was extremely high. The second column in Table 10 indicates the number of individual experiments from which the data were taken. In column 6 (Percentage Mean Deviation), only 5 out of 16 figures are over 10%, 3 of these being for 10^{-4}M IAA, where the results of one experiment differed rather widely from the other two. In Fig.27 the mean uptake values for the series of IAA concentrations are plotted against time. The initial rate of uptake was relatively high, but subsequent uptake over 5 hours was fairly linear, except at the highest concentration of IAA (10^{-4}M) where some decrease in the rate of uptake over time was recorded. In Fig.28 the rate of uptake has been plotted against concentration. A fairly

TABLE 10

IAA	No. of Expts.	TIME	MEAN UPTAKE) cpm	M.D.	% M.D.	RATE
10^{-5}M	5	1 hr	100	7.8	7.80	
		2 hr	160	11.6	7.25	48.00
		4 hr	253	37.6	14.86	
		6 hr	343	34.4	10.00	
2.5×10^{-5}M	2	1 hr	347	9.0	2.59	
		2 hr	466	26.0	5.58	158.98
		4 hr	842	94.0	11.16	
		6 hr	1119	99.0	8.85	
5×10^{-5}M	9	1 hr	621	51.0	8.21	
		2 hr	1023	102.0	9.97	298.44
		4 hr	1569	89.2	5.69	
		6 hr	2146	159.2	7.42	
10^{-4}M	3	1 hr	1130	150.3	13.30	
		2 hr	2098	459.7	21.90	552.54
		4 hr	3237	421.0	13.00	
		6 hr	3959	296.7	7.50	

Uptake of IAA - C^{14} by segments of the mesocotyl of Zea mays, represented in CPM per 5 segments. M.D. = Mean Deviation.

FIG. 27: UPTAKE OF IAA-C^{14}

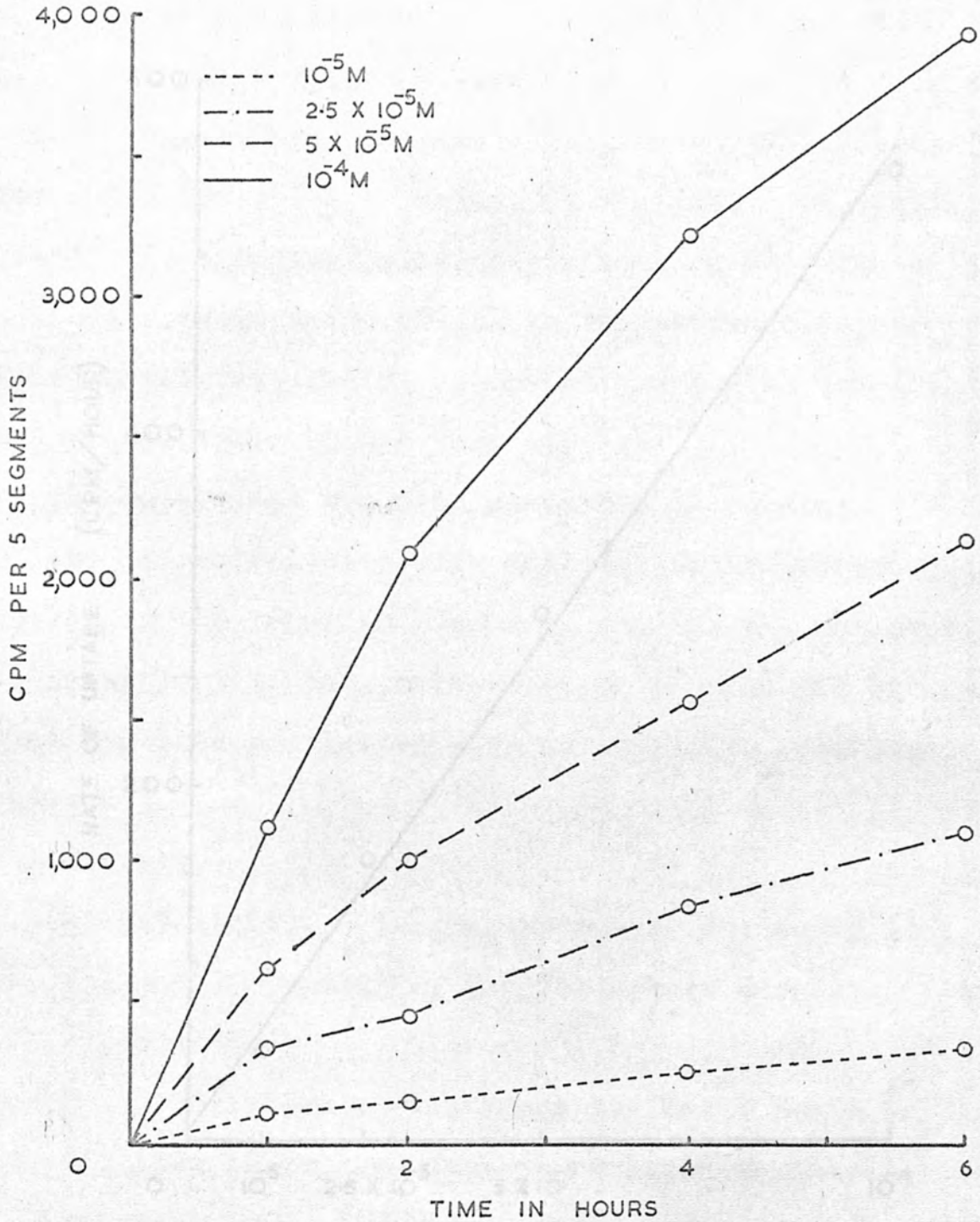
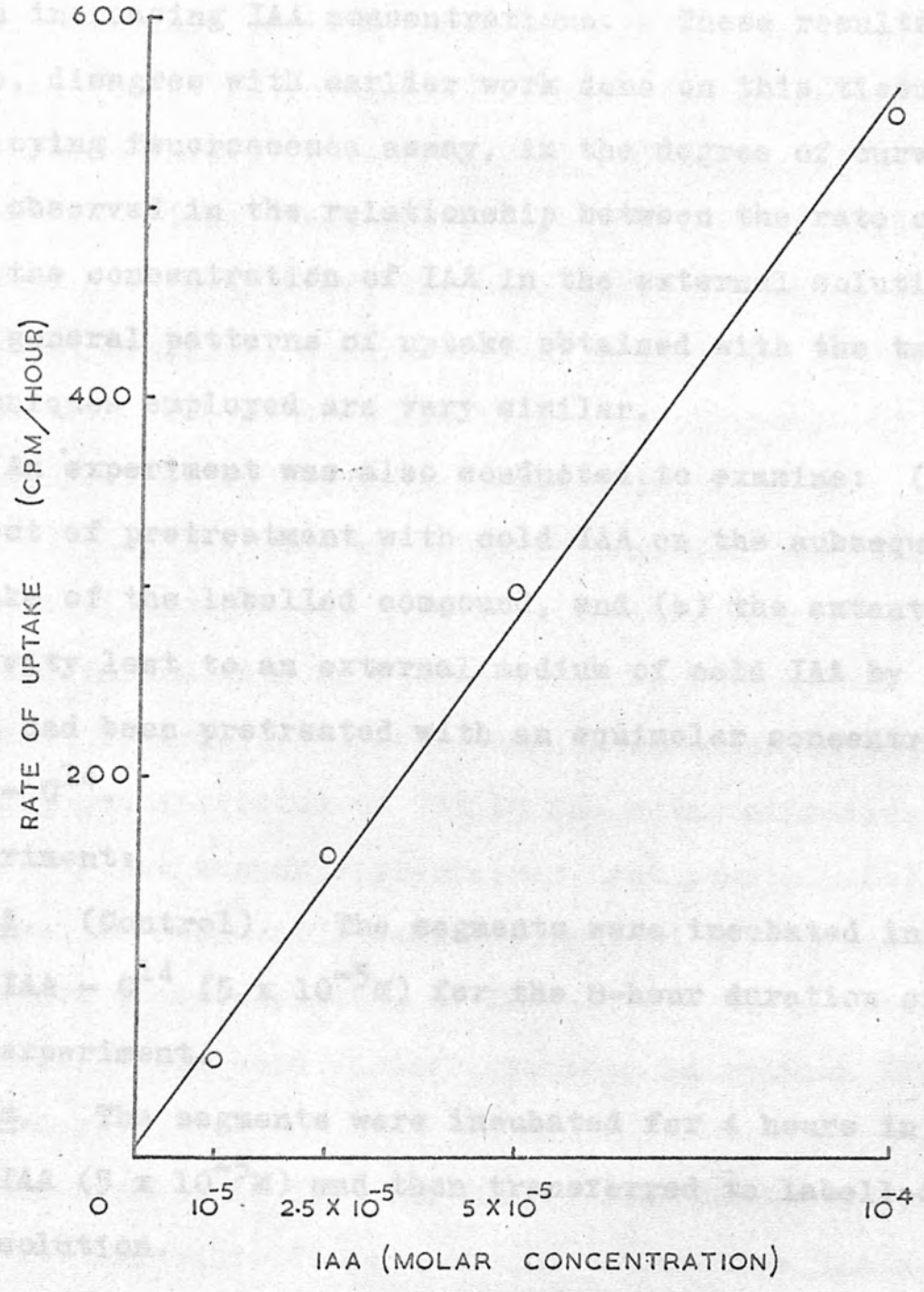


FIG. 28


600
400
200
0
RATE OF UPTAKE (CPM/HOUR)
10^{-5}
2.5×10^{-5}
5×10^{-5}
10^{-4}
IAA (MOLAR CONCENTRATION)

linear relationship is obtained over the range of IAA concentrations examined (10^{-5}M to 10^{-4}M), although a tendency is discernible for the curve to slope off slightly with increasing IAA concentrations. These results, therefore, disagree with earlier work done on this tissue employing fluorescence assay, in the degree of curvilinearity observed in the relationship between the rate of uptake and the concentration of IAA in the external solution. The general patterns of uptake obtained with the two techniques employed are very similar.

An experiment was also conducted to examine: (a) the effect of pretreatment with cold IAA on the subsequent uptake of the labelled compound, and (b) the extent of activity lost to an external medium of cold IAA by segments that had been pretreated with an equimolar concentration of IAA - C^{14}.

Experiment:

A. (Control). The segments were incubated in IAA - C^{14} (5×10^{-5}M) for the 8-hour duration of the experiment.

B. The segments were incubated for 4 hours in "cold" IAA (5×10^{-5}M) and then transferred to labelled IAA solution.

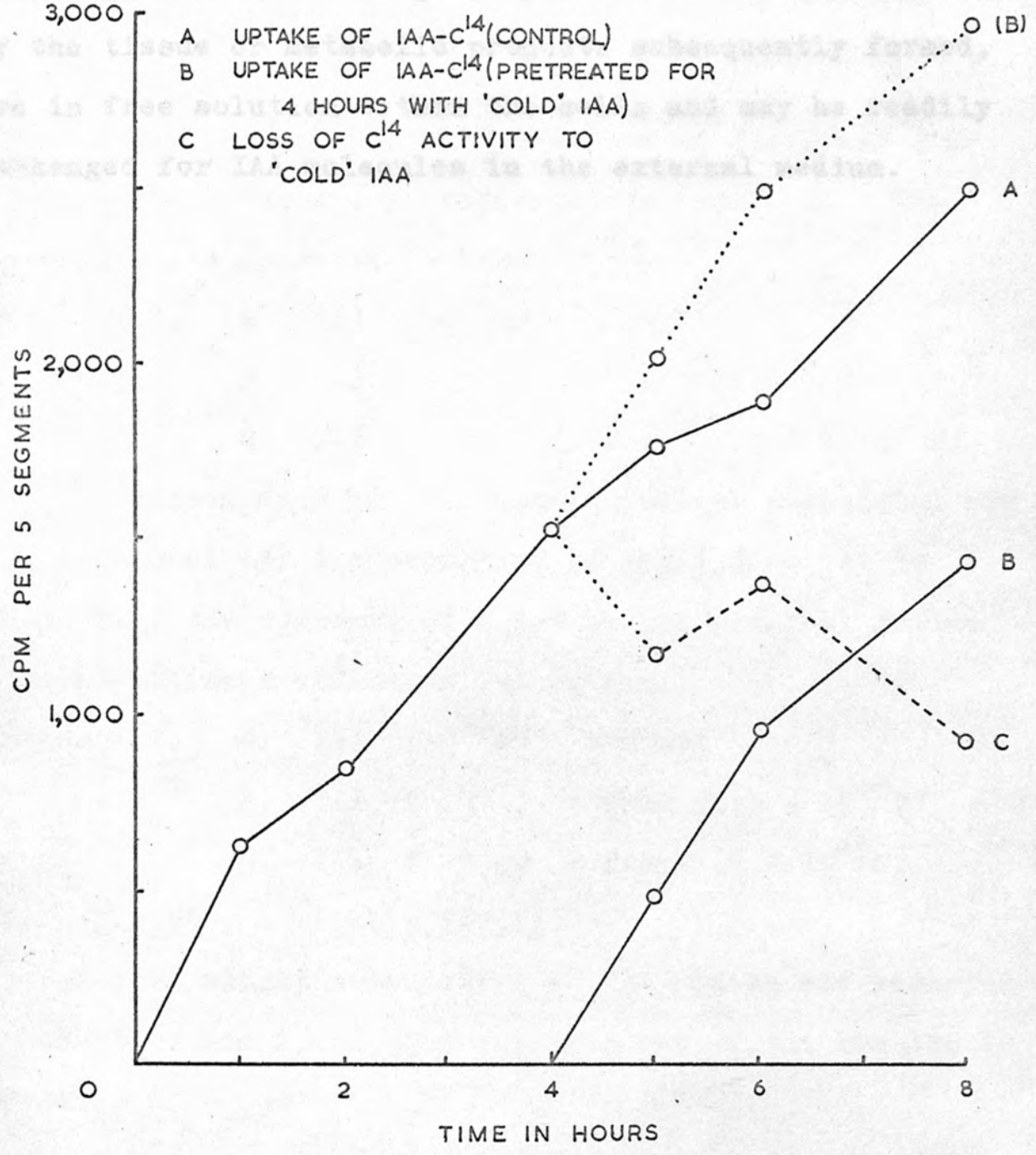
C. The segments were pretreated for 4 hours with IAA - C^{14} (5×10^{-5}M) and then transferred to a similar concentration of cold IAA.

Batches of segments from each treatment were removed at intervals and assayed for activity. The results are presented in Fig. 29 (Table XXVII)

It is evident that pretreatment with "cold" IAA for 4 hours does not affect the subsequent uptake of the labelled compound, over a further 4 hours by Zea mesocotyl segments. The pattern and magnitude of uptake of IAA-C^{14} by the pretreated segments is identical with that exhibited by the control batch of segments over the first 4 hours of incubation. It must follow, therefore, that the decline in the rate of uptake of IAA - C^{14} by the control segments between 4 - 8 hours can be ascribed to (a) the falling concentration of IAA in the external medium as uptake by the segments progresses, and possibly (b) a decrease in measurable radioactivity in the tissue due to oxidative decarboxylation of IAA - C^{14}. This latter possibility has been further examined in section XVI of this chapter.

The results of this experiment also indicate that when segments incubated for 4 hours in radioactive IAA are

FIG. 29


A UPTAKE OF IAA-C14 (CONTROL)
B UPTAKE OF IAA-C14 (PRETREATED FOR 4 HOURS WITH 'COLD' IAA)
C LOSS OF C14 ACTIVITY TO 'COLD' IAA
(B)
A
B
C
3,000
2,000
1,000
0
2
4
6
8
CPM PER 5 SEGMENTS
TIME IN HOURS

transferred to a solution of non-radioactive IAA of the same initial concentration, 40% of the C^{14} activity is lost to the external solution over a further period of 4 hours. This suggests that a large proportion of the IAA absorbed by the tissue or metabolic products subsequently formed, are in free solution within the cells and may be readily exchanged for IAA molecules in the external medium.

VIII

MATERIAL: Zea mays - mesocotyl

METHOD: Fluorescence assay

GROWTH-SUBSTANCES: IAA + 2,4-D

IAA + NMSP

The effect of various concentrations of 2,4-D and NMSP on IAA uptake by mesocotyl segments was examined. The experiments are described below:

Experiment 1. A. IAA (10^{-5}M) Control

B. IAA (") + 2,4-D (10^{-5}M)

C. IAA (") + 2,4-D (2.5×10^{-5}M)

The fluorescence data and the uptake figures calculated for the IAA control (A) are presented in Table 11. It is evident that the presence of 2,4-D in the external medium has no significant effect on IAA uptake.

Experiment 2. A. IAA (10^{-5}M) Control

B. IAA (") + NMSP (2.5×10^{-5}M)

C. IAA (") + 2,4-D (5×10^{-5}M)

Data: Fig.30 (Table XXVIII)

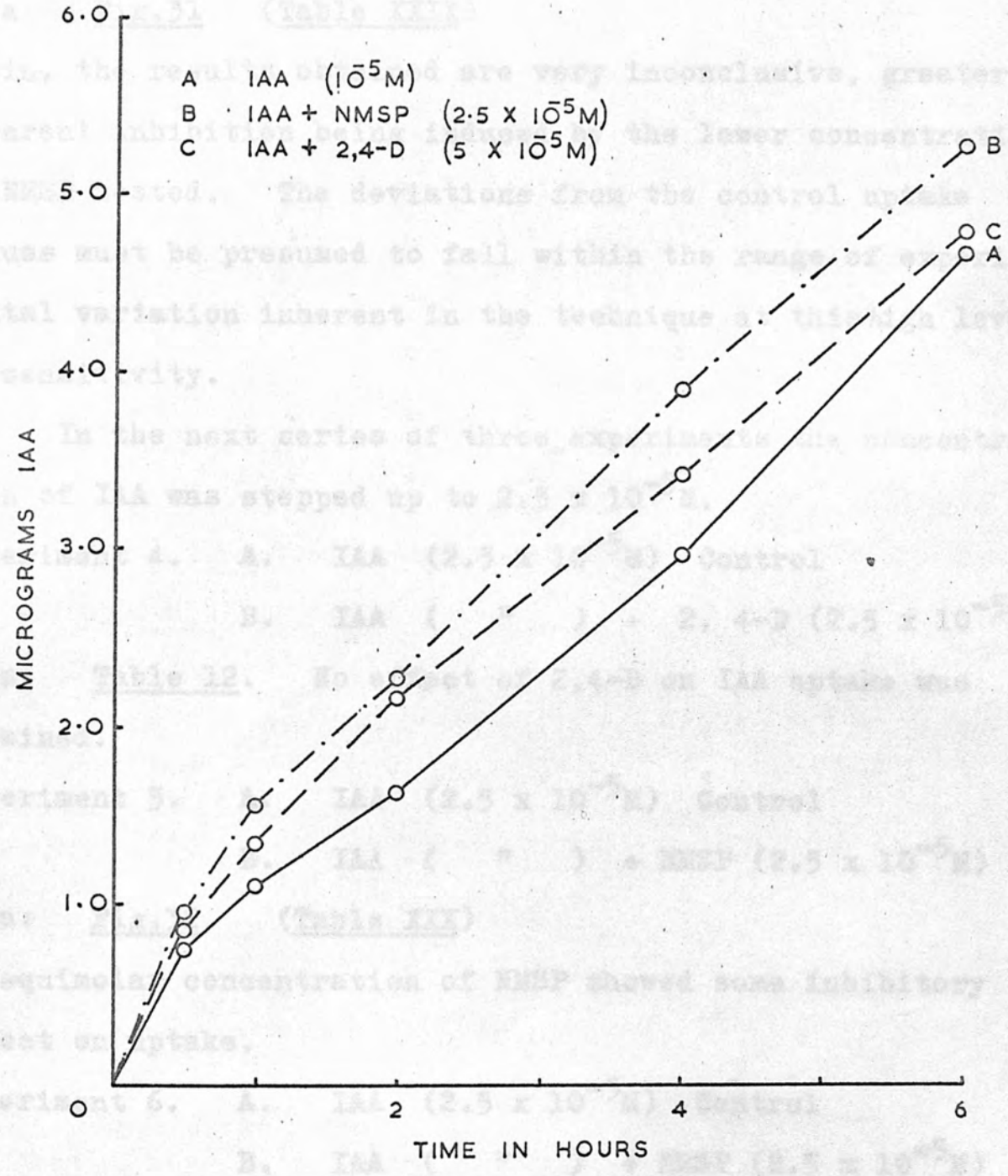
Although some slight stimulation of IAA uptake was recorded for both NMSP and 2,4-D, the significance of the results is doubtful.

TABLE 11

TIME	FLUORESCENCE INTENSITY			UPTAKE OF IAA
	A	B	C	A
30 m	33.02	32.5	35.0	____
1 hr	31.8	30.5	33.8	0.58
2 hr	30.5	29.0	31.7	1.16
4 hr	27.8	26.0	27.5	2.31
7 hr	21.0	19.5	21.5	5.01

Effect of 2,4-D on the uptake of IAA by ZEA mesocotyl segments.
A = IAA (10^{-5}M); B = IAA (10^{-5}M) + 2,4-D (10^{-5}M);
C = IAA (10^{-5}M) ± 2,4-D (2.5×10^{-5}M).

FIG. 30


A IAA (10^{-5}M)
B IAA + NMSP (2·5 X 10^{-5}M)
C IAA + 2,4-D (5 X 10^{-5}M)
MICROGRAMS IAA
TIME IN HOURS
0
1·0
2·0
3·0
4·0
5·0
6·0
2
4
6
A
B
C

Experiment 3. A. IAA (10^{-5}M) Control

B. IAA (") + NMSP (10^{-5}M)

C. IAA (") + NMSP (5×10^{-5}M)

Data: Fig.31 (Table XXIX)

Again, the results obtained are very inconclusive, greater apparent inhibition being induced by the lower concentration of NMSP tested. The deviations from the control uptake values must be presumed to fall within the range of experimental variation inherent in the technique at this high level of sensitivity.

In the next series of three experiments the concentration of IAA was stepped up to 2.5×10^{-5}M.

Experiment 4. A. IAA (2.5×10^{-5}M) Control

B. IAA (") + 2, 4-D (2.5×10^{-5}M)

Data: Table 12. No effect of 2,4-D on IAA uptake was obtained.

Experiment 5. A. IAA (2.5×10^{-5}M) Control

B. IAA (") + NMSP (2.5×10^{-5}M)

Data: Fig.32 (Table XXX)

An equimolar concentration of NMSP showed some inhibitory effect on uptake.

Experiment 6. A. IAA (2.5×10^{-5}M) Control

B. IAA (") + NMSP (2.5×10^{-5}M)

C. IAA (") + 2,4-D (5×10^{-5}M).

Data: Table 13

FIG. 31

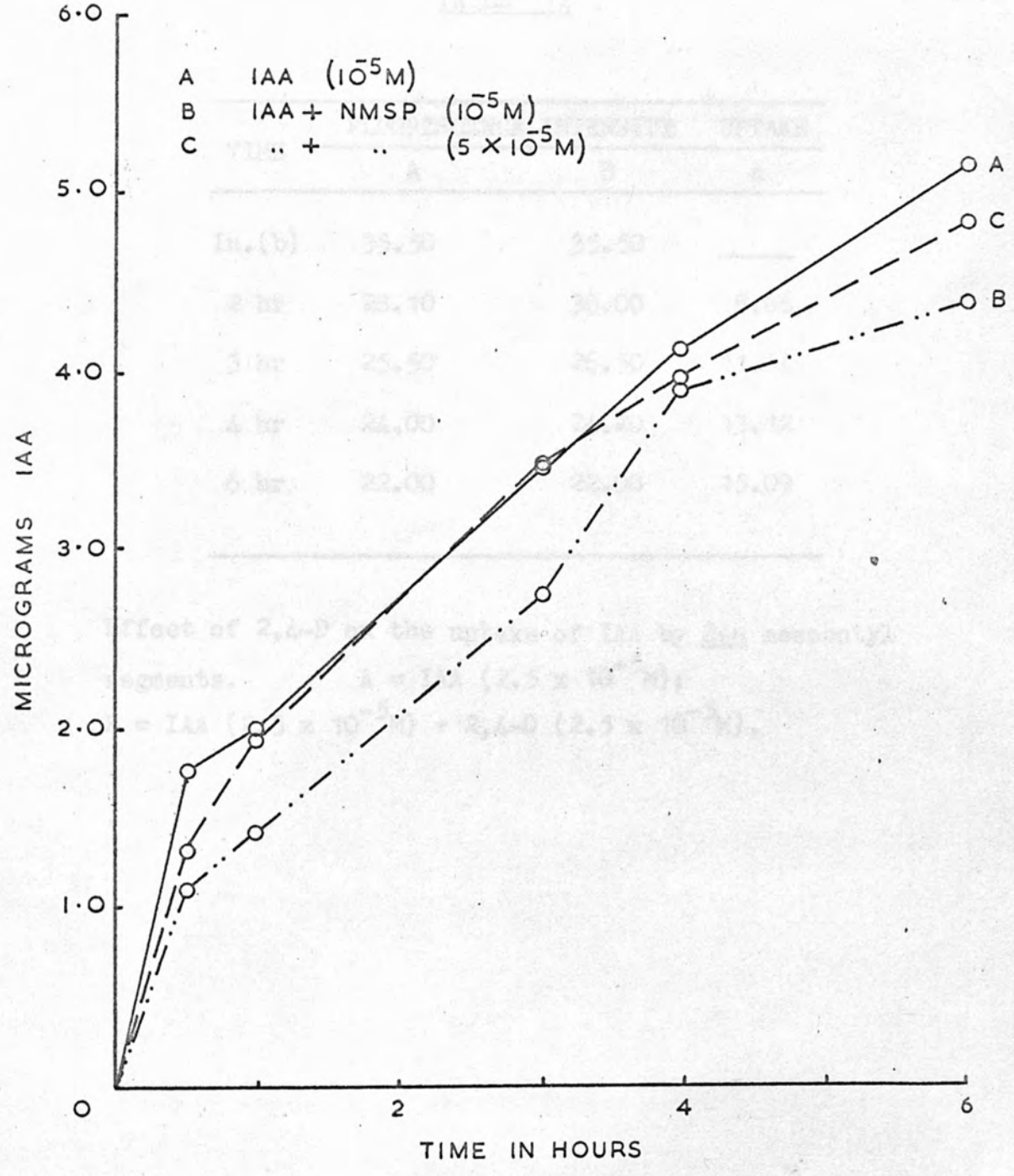

A IAA (10^{-5}M)
B IAA + NMSP (10^{-5}M)
C .. + .. (5×10^{-5}M)
MICROGRAMS IAA
TIME IN HOURS
6·0
5·0
4·0
3·0
2·0
1·0
0
2
4
6
A
C
B

TABLE 12

TIME	FLUORESCENCE INTENSITY		UPTAKE
	A	B	A
In.(b)	35.50	35.50	____
2 hr	28.10	30.00	8.66
3 hr	25.50	26.50	11.54
4 hr	24.00	24.20	13.12
6 hr	22.00	22.00	15.09

Effect of 2,4-D on the uptake of IAA by Zea mesocotyl segments. A = IAA (2.5×10^{-5}M); B = IAA (2.5×10^{-5}M) + 2,4-D (2.5×10^{-5}M).

FIG. 32

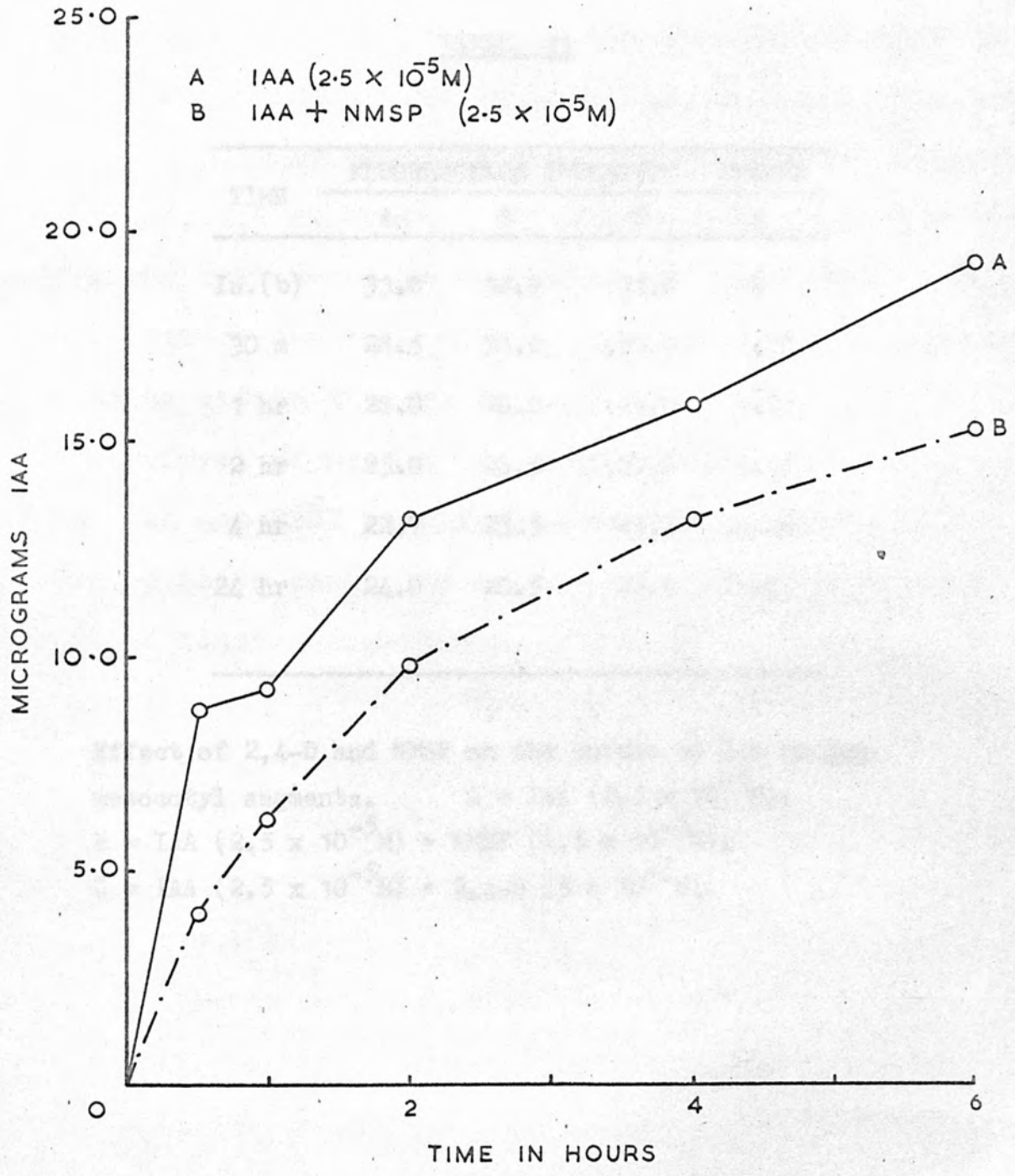

25·0
20·0
15·0
10·0
5·0
0
2
4
6
A IAA (2·5 × 10⁻⁵M)
B IAA + NMSP (2·5 × 10⁻⁵M)
A
B
MICROGRAMS IAA
TIME IN HOURS

TABLE 13

TIME	FLUORESCENCE INTENSITY			UPTAKE
	A	B	C	A
In.(b)	33.0	32.0	31.0	0
30 m	28.5	31.0	30.0	5.36
1 hr	28.0	28.0	29.0	5.93
2 hr	25.0	26.5	27.5	9.11
4 hr	22.7	23.5	23.5	11.40
24 hr	24.0	20.5	27.5	25.05

Effect of 2,4-D and NMSP on the uptake of IAA by Zea mesocotyl segments. A = IAA (2.5×10^{-5}M); B = IAA (2.5×10^{-5}M) + NMSP (2.5×10^{-5}M); C = IAA (2.5×10^{-5}M) + 2,4-D (5×10^{-5}M).

Repeating the previously tried concentration of NMSP this time gave negative results. 2,4-D at 5 x 10^{-5}M was also without any effect.

One may therefore assume with some certainty from the above experiments that neither 2,4-D nor NMSP have any effect on the uptake of IAA by maize mesocotyl segments. In the case of 2,4-D, this supports the results obtained with the Avena coleoptile segments. In the case of NHSP, the above results can only serve to emphasize the fact that the fluctuating inhibitions obtained with Avena tissue either must be taken to be within the experimental error of the experiments and, therefore, of negligible significance, or an interaction between IAA and NMSP peculiar to the latter tissue, the pattern of which cannot be determined using the present methods of fluorescence assay.

IX (a)

MATERIAL: Zea mays - mesocotyl

METHOD: Fluorescence assay

GROWTH-SUBSTANCES: IAA + N - 1 - naphthyl - phthalamic acid (NPA)

α - Naphthylphthalamic acid (NPA) has been found by many workers to exhibit anti-auxin properties in some of the growth reactions presumably controlled by auxins. An attempt was made to examine its effects on the uptake of IAA. It was found, however, that although NPA itself does not fluoresce, it quenches the fluorescence of IAA fairly heavily. A correction for quenching might be effectively applied to the initial fluorescence readings, as was applied to all the readings obtained with added NMSP, but in this case, as the quenching was considerably greater and may be assumed to change significantly with changing concentrations of the two compounds in the external medium during uptake, it was considered that any arbitrary corrections applied to the values would have no reasonable validity. The drop in fluorescence intensity during uptake when plotted against time gave reasonably linear graphs. The slopes of these curves were, therefore, calculated from the fluorescence values obtained between 1 hour and 6 hours of incubation, and these slopes were employed for analyses of interactions

between the two compounds.

The results are presented in Figs. 33-37, (Tables XXXI - XXXV). Percentage inhibition or stimulation of the rate of IAA uptake (in terms of decreasing fluorescence intensity) induced by NPA, is plotted in Fig.38 against the concentration of NPA.

It may be seen from the results that NPA at all concentrations stimulated the uptake of IAA at 10^{-5}M. The experiment with 2.5 x 10^{-5}M IAA was repeated, and some considerable variation was recorded. In Fig.38 the mean values from the two experiments are represented and indicate stimulation of IAA (2.5 x 10^{-5}M) uptake by NPA over the range of concentrations tested. With the two higher concentrations of IAA (5 x 10^{-5}M and 10^{-4}M), NPA at low concentrations stimulated uptake but at higher concentrations showed slightly inhibitory, or no effects.

There is, therefore, strong evidence of positive interaction between the two compounds during the uptake and accumulation of IAA by the tissue. As might be expected, this is more pronounced at low interacting concentrations of IAA and NPA, and the effect is reduced or completely overcome by increasing concentrations of one or both compounds.

FIG. 33

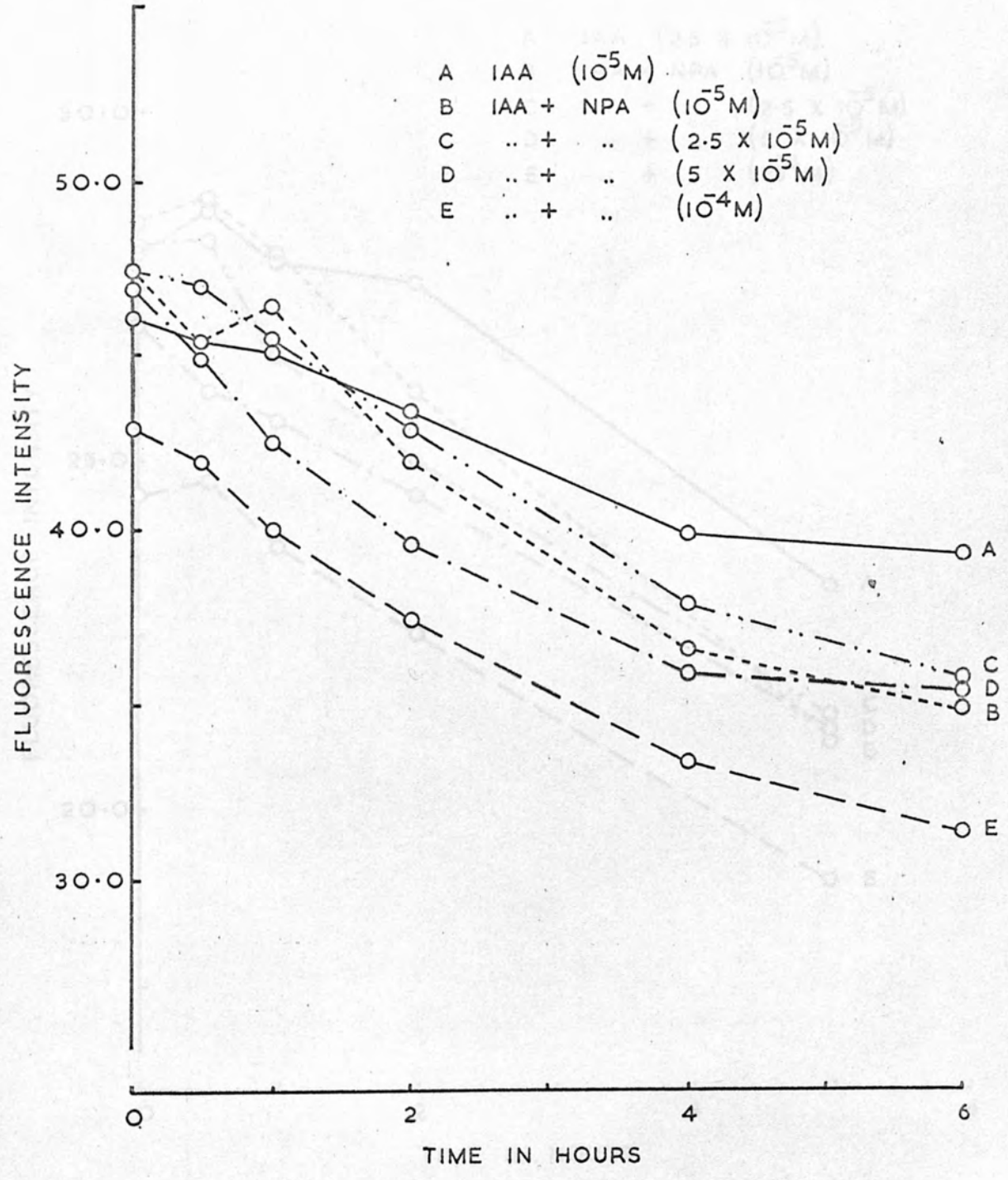

A IAA (10^{-5}M)
B IAA + NPA (10^{-5}M)
C .. + .. (2.5×10^{-5}M)
D .. + .. (5×10^{-5}M)
E .. + .. (10^{-4}M)
50·0
40·0
30·0
FLUORESCENCE INTENSITY
0
2
4
6
TIME IN HOURS
A
C
D
B
E

FIG. 34

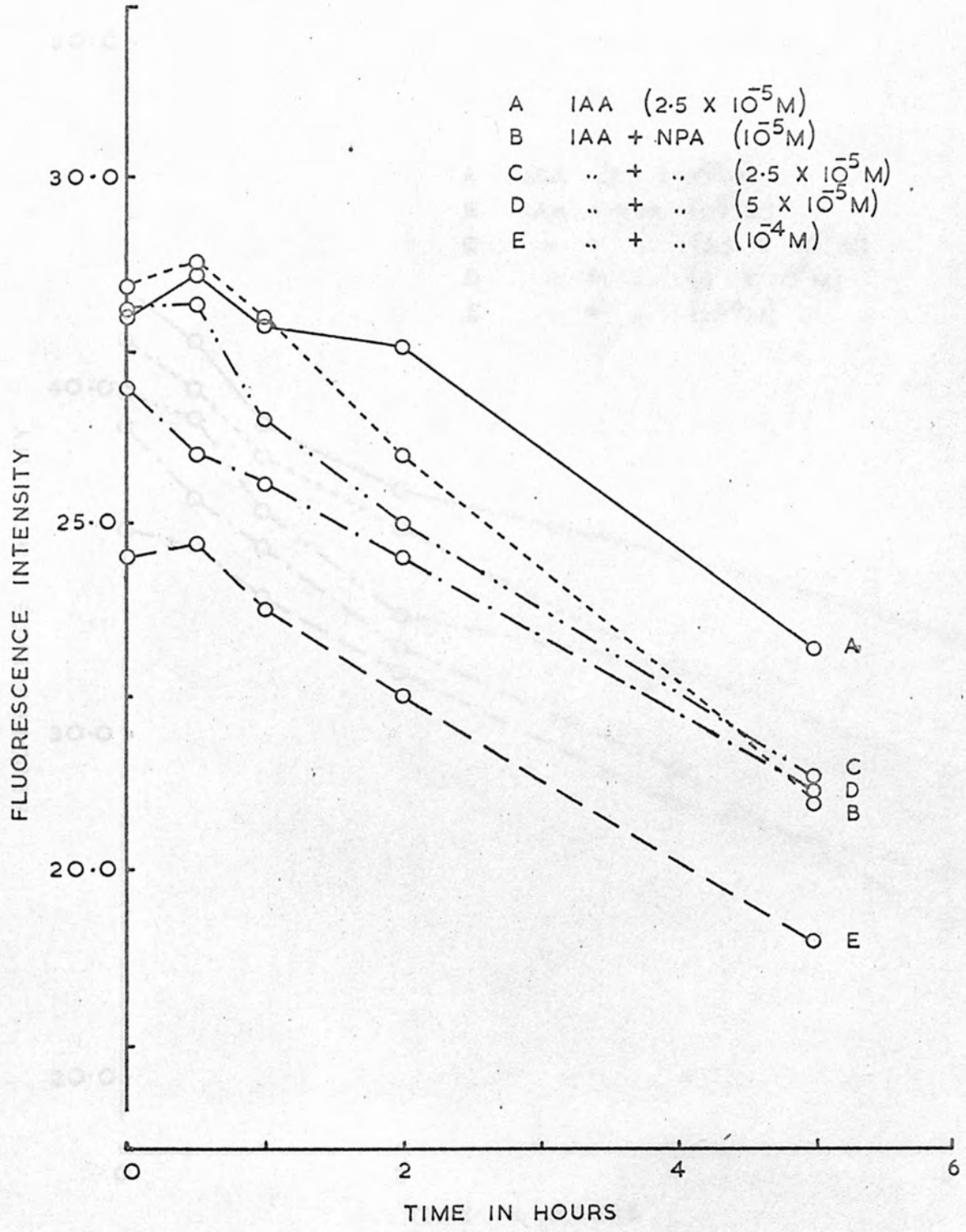

A IAA (2·5 X 10⁻⁵M)
B IAA + NPA (10⁻⁵M)
C .. + .. (2·5 X 10⁻⁵M)
D .. + .. (5 X 10⁻⁵M)
E .. + .. (10⁻⁴M)
30·0
25·0
20·0
FLUORESCENCE INTENSITY
0
2
4
6
TIME IN HOURS
A
C
D
B
E

FIG. 35

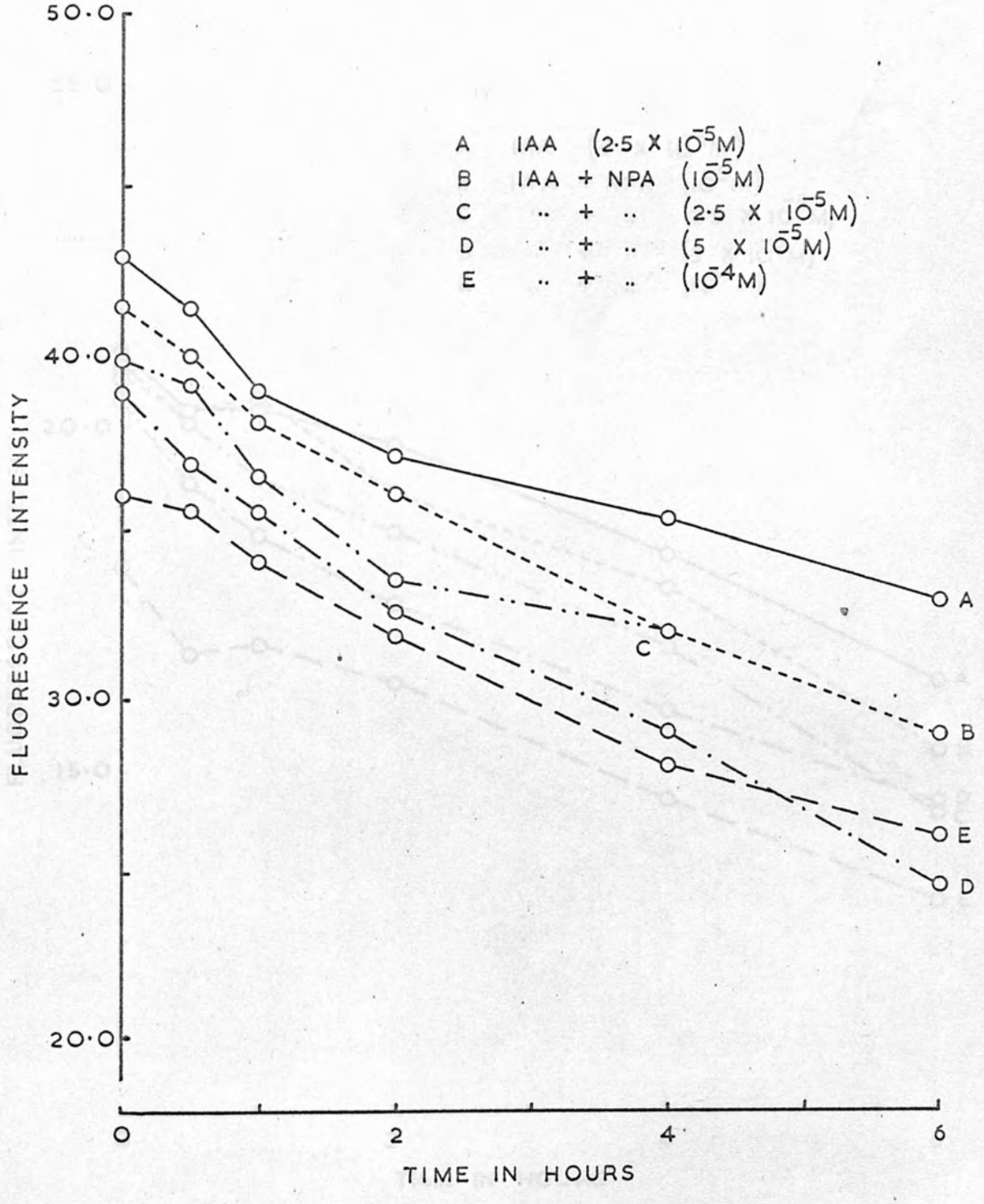

A IAA (2·5 X 10⁻⁵M)
B IAA + NPA (10⁻⁵M)
C .. + .. (2·5 X 10⁻⁵M)
D .. + .. (5 X 10⁻⁵M)
E .. + .. (10⁻⁴M)
50·0
40·0
30·0
20·0
FLUORESCENCE INTENSITY
0
2
4
6
TIME IN HOURS
A
B
C
D
E

FIG. 36

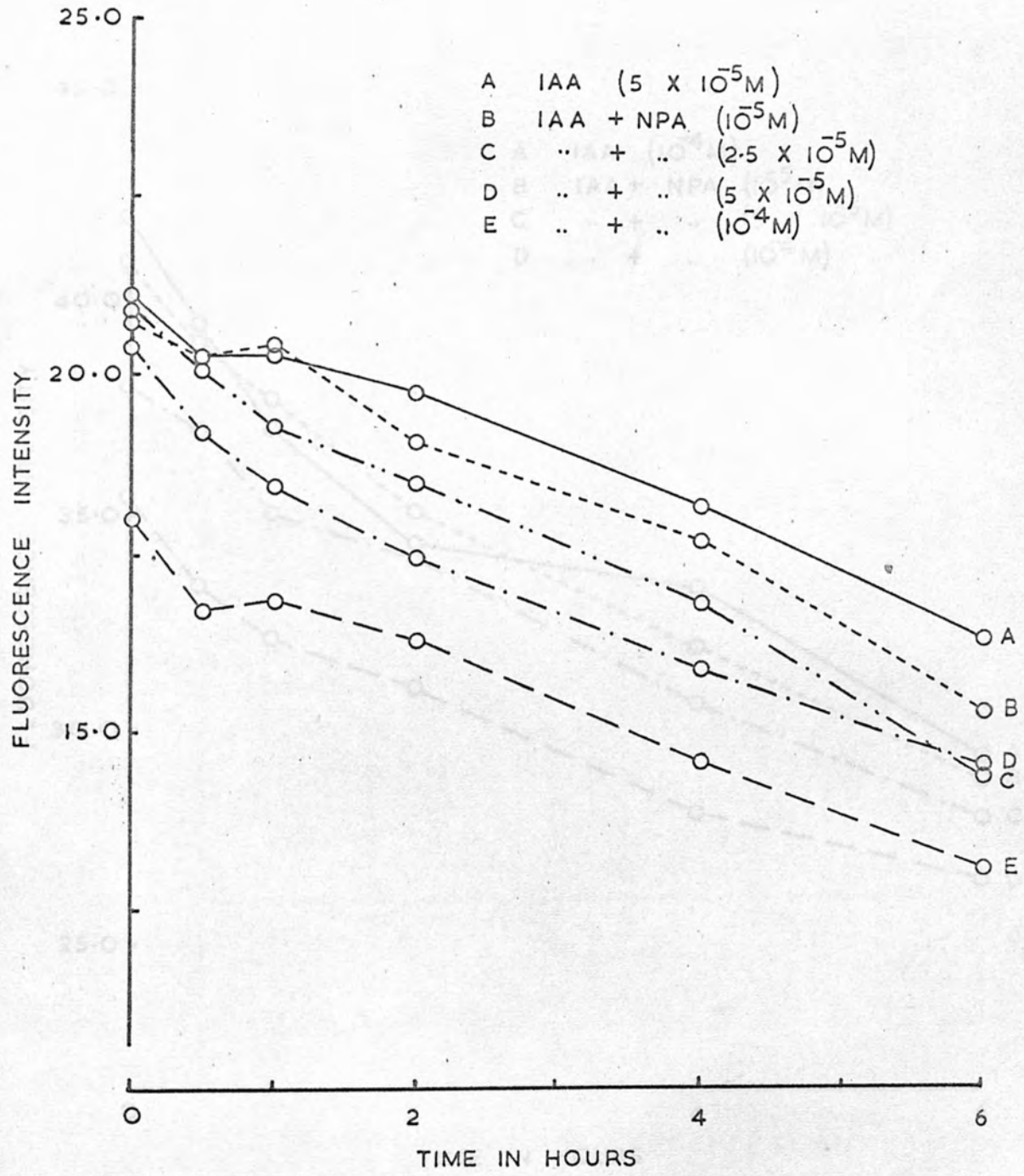

A IAA (5 X 10⁻⁵M)
B IAA + NPA (10⁻⁵M)
C .. + .. (2·5 X 10⁻⁵M)
D .. + .. (5 X 10⁻⁵M)
E .. + .. (10⁻⁴M)
25·0
20·0
15·0
FLUORESCENCE INTENSITY
0
2
4
6
TIME IN HOURS
A
B
D
C
E

FIG. 37

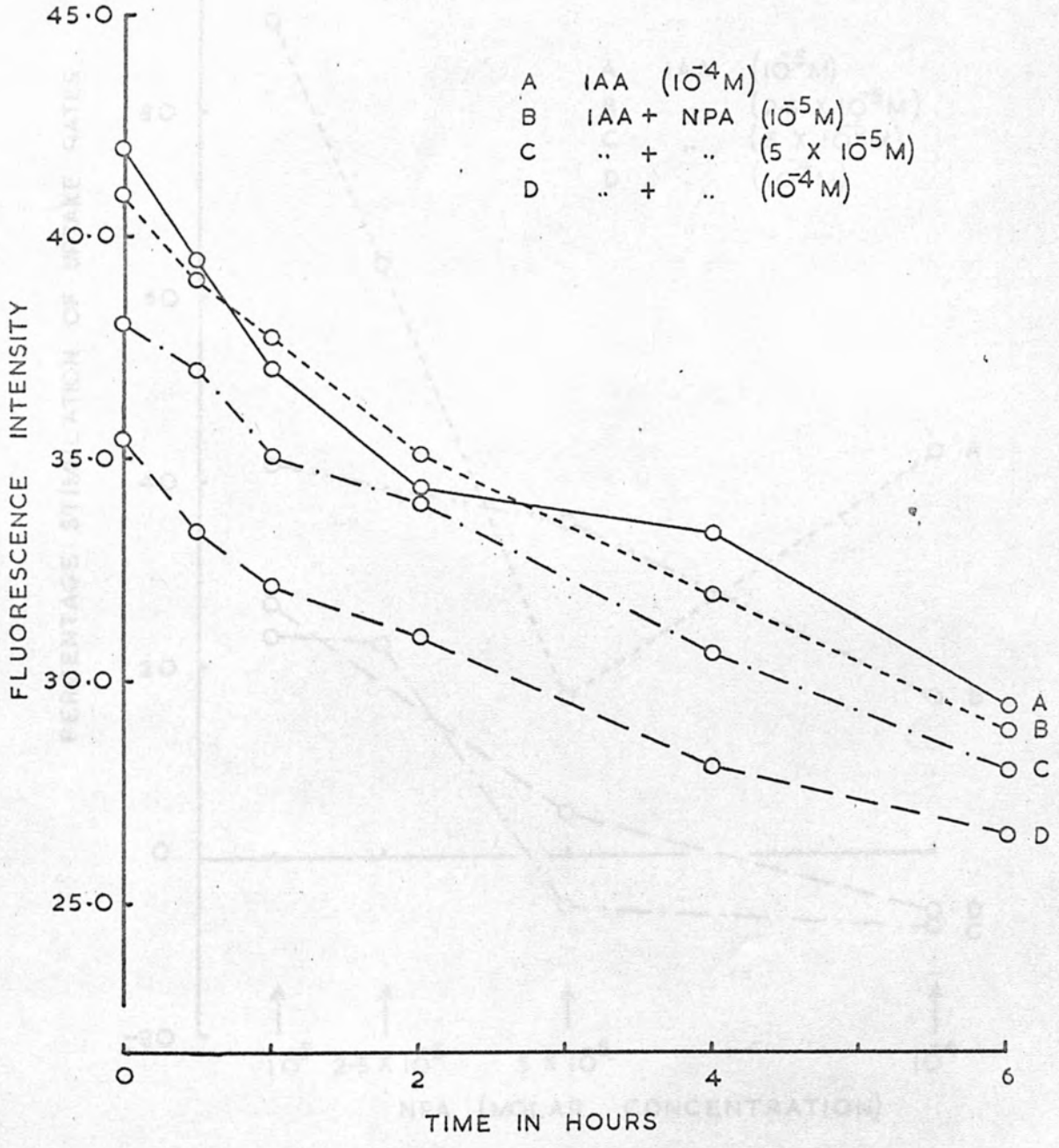
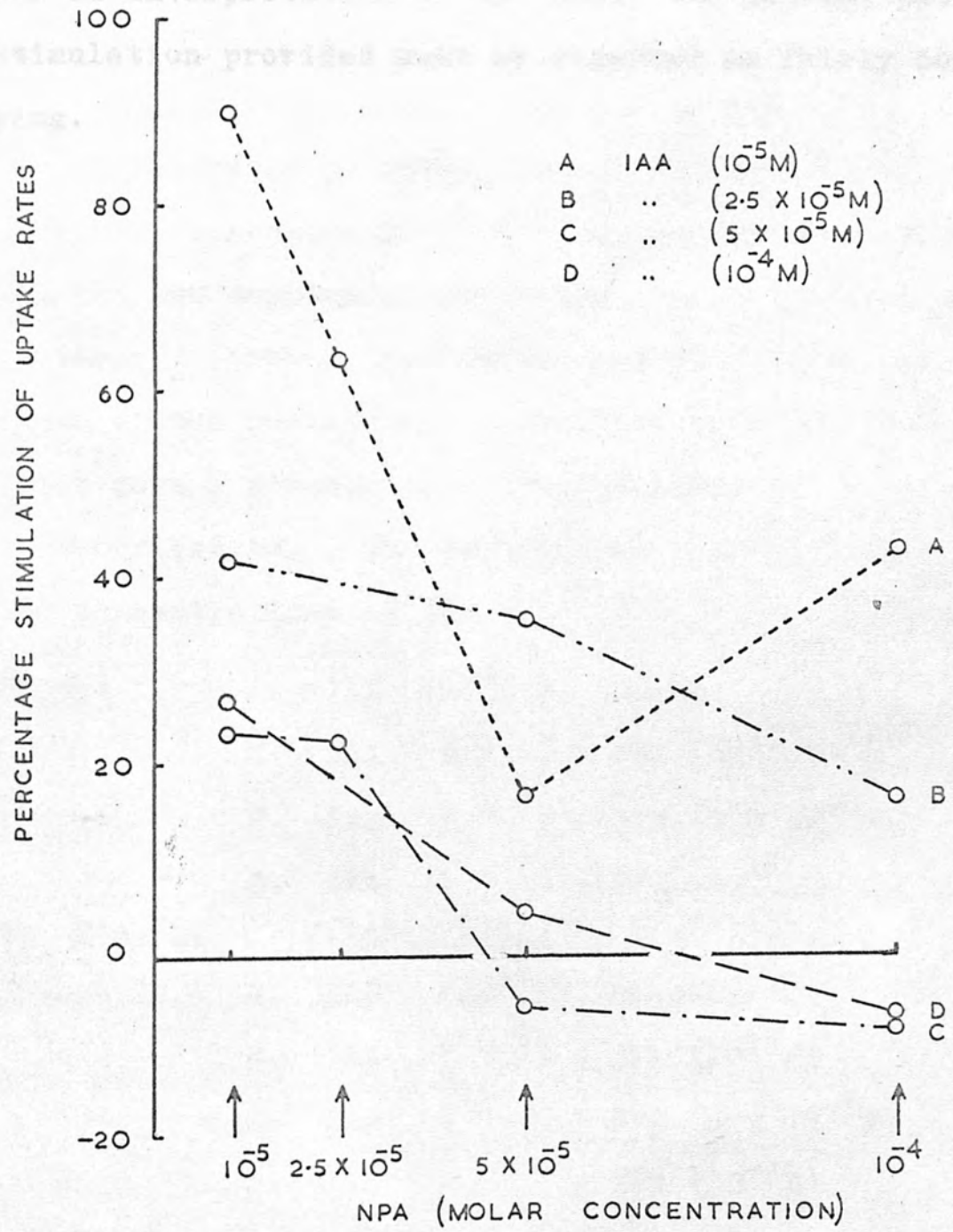

45·0
40·0
35·0
30·0
25·0
FLUORESCENCE INTENSITY
A IAA (10^{-4}M)
B IAA + NPA (10^{-5}M)
C .. + .. (5 X 10^{-5}M)
D .. + .. (10^{-4}M)
A
B
C
D
0
2
4
6
TIME IN HOURS

FIG. 38


100
80
60
40
20
0
-20
PERCENTAGE STIMULATION OF UPTAKE RATES
A IAA (10^{-5}M)
B .. (2.5×10^{-5}M)
C .. (5×10^{-5}M)
D .. (10^{-4}M)
A
B
D
C
10^{-5}
2.5×10^{-5}
5×10^{-5}
10^{-4}
NPA (MOLAR CONCENTRATION)

In spite of the limited number of experiments conducted and the hurdles imposed by the quenching effects of NPA on interpretation of the data, the general pattern of stimulation provided must be regarded as fairly convincing.

IX (b)

MATERIAL: Zea mays - mesocotyl

METHOD: Radioactivity assay

GROWTH-SUBSTANCES. IAA - C^{14} + NPA

N-1-naphthyl-phthalamic acid (NPA), employing the methods of fluorescence assay, apparently stimulated the uptake of low concentrations of IAA, the degree of interaction between the two compounds decreasing with increasing concentrations. However, the limitations imposed on the interpretation of the results by the difficulties inherent in the technique gave a somewhat doubtful validity to the conclusions arrived at. The experiments were therefore repeated for two concentrations of IAA - C^{14} (10^{-5}M and 10^{-4}M).

Experiment 1. A. IAA (10^{-5}M) Control

B. IAA (") + NPA (10^{-5}M)

C. IAA (") + NPA (5×10^{-5}M)

D. IAA (") + NPA (10^{-4}M).

Data: Fig. 39 (Table XXXVI)

Experiment 2. A. IAA (10^{-4}M) Control

B. IAA (") + NPA (10^{-5}M)

C. IAA (") + NPA (5×10^{-5}M)

D. IAA (") + NPA (10^{-4}M)

Data: Fig.40 (Table XXXVII)

FIG. 39

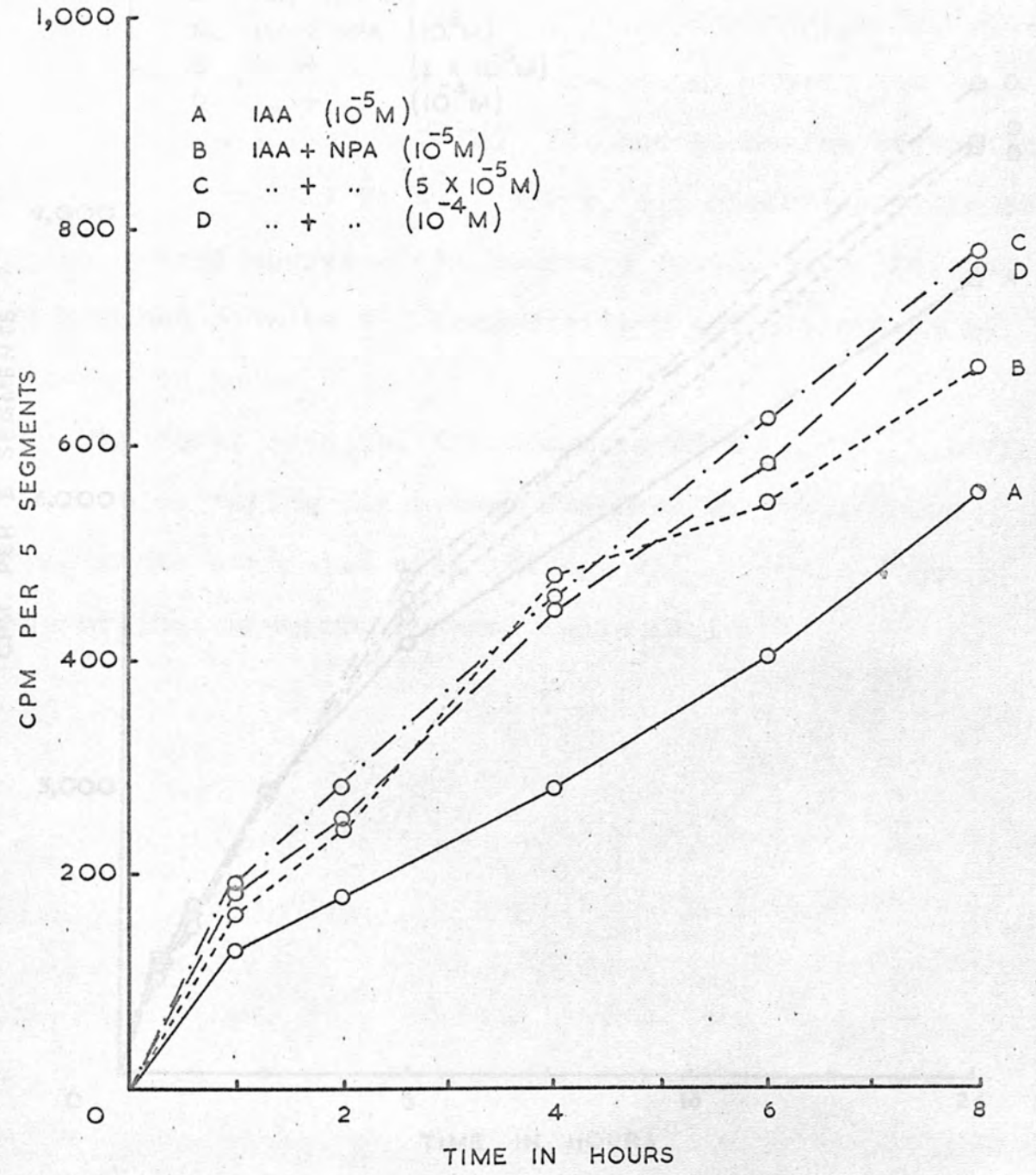
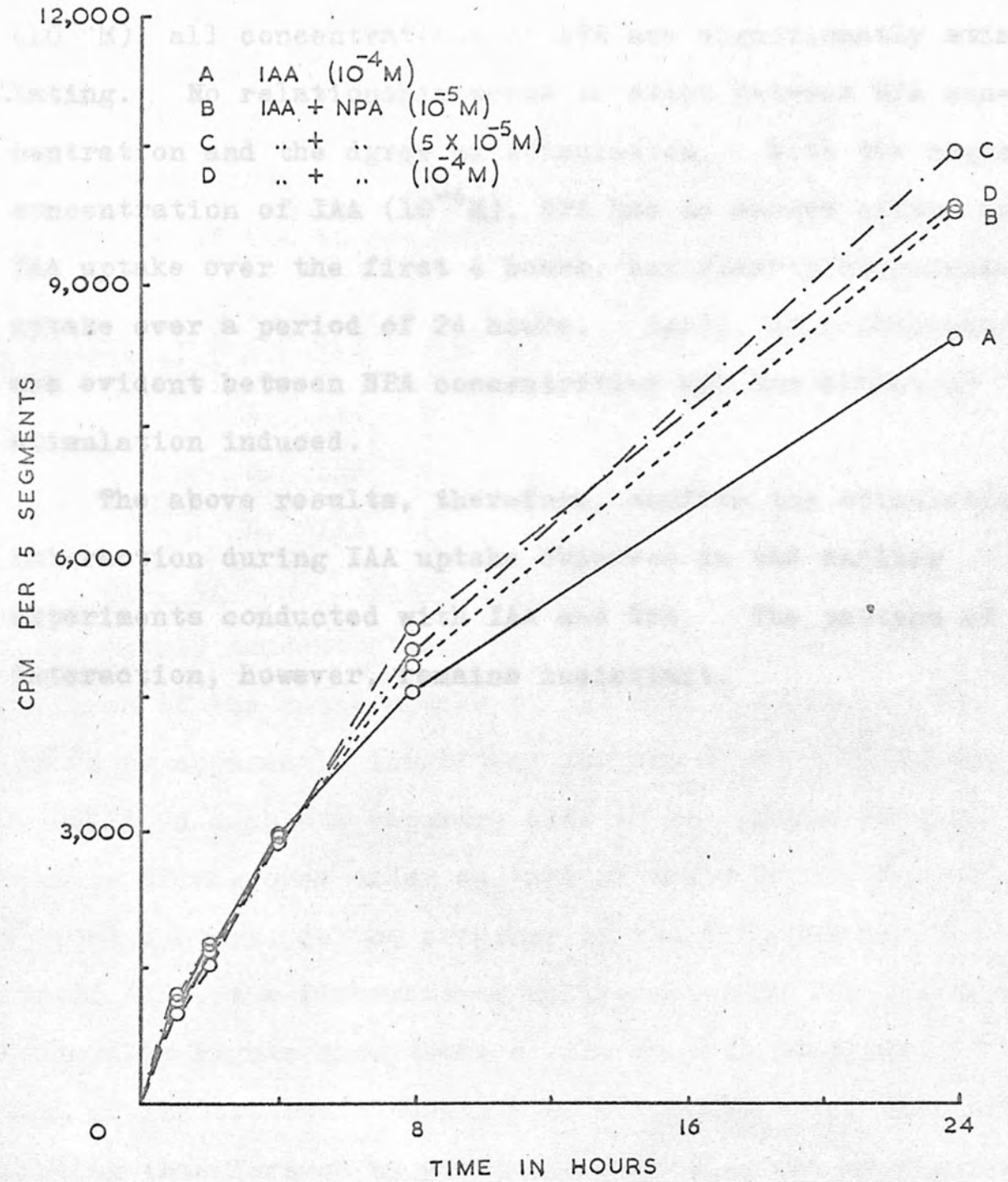

1,000
800
600
400
200
0
CPM PER 5 SEGMENTS
A IAA (10^{-5}M)
B IAA + NPA (10^{-5}M)
C .. + .. (5×10^{-5}M)
D .. + .. (10^{-4}M)
C
D
B
A
2
4
6
8
TIME IN HOURS

FIG. 40


12,000
9,000
6,000
3,000
0
CPM PER 5 SEGMENTS
A IAA (10^{-4}M)
B IAA + NPA (10^{-5}M)
C .. + .. (5×10^{-5}M)
D .. + .. (10^{-4}M)
C
D
B
A
8
16
24
TIME IN HOURS

It is evident that NPA does indeed stimulate the uptake of IAA. With the lower concentration of IAA (10^{-5}M), all concentrations of NPA are significantly stimulating. No relationship seems to exist between NPA concentration and the dgree of stimulation. With the higher concentration of IAA (10^{-4}M), NPA has no marked effect on IAA uptake over the first 4 hours, but clearly stimulates uptake over a period of 24 hours. Again, no relationship was evident between NPA concentration and the extent of stimulation induced.

The above results, therefore, confirm the stimulating interaction during IAA uptake observed in the earlier experiments conducted with IAA and NPA. The pattern of interaction, however, remains indistinct.

X (a)

MATERIAL: Zea mays - mesocotyl

METHOD: Fluorescence assay

GROWTH-SUBSTANCES: IAA + TIBA

Triiodobenzoic acid (TIBA) has no fluorescent properties of its own and does not interfere significantly with the fluorescence of IAA at concentrations employed in the present study.

Experiment 1. A. IAA (10^{-5}M) Control

B. IAA (") + TIBA (10^{-5}M)

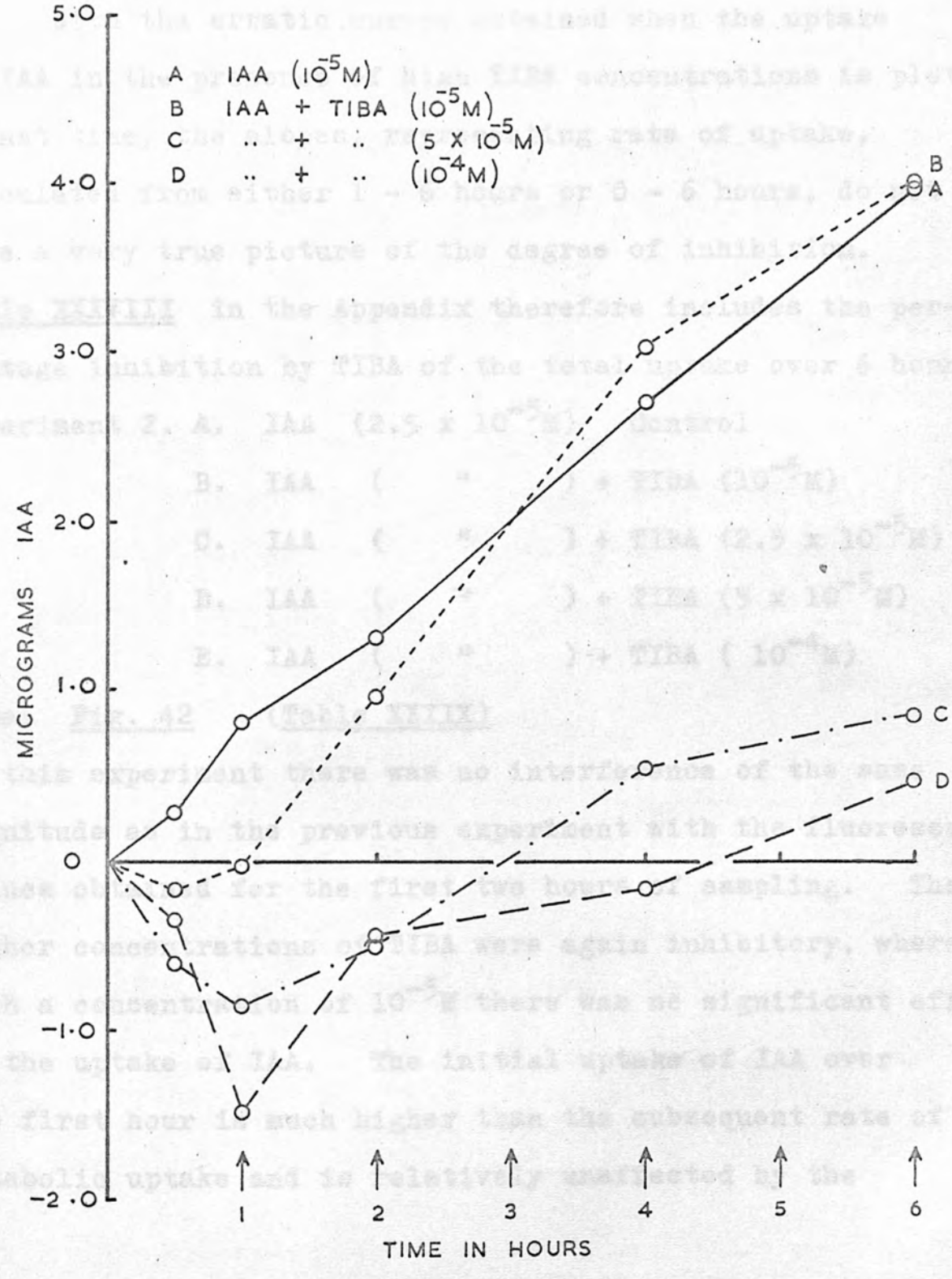
C. IAA (") + TIBA (5×10^{-5}M)

D. IAA (") + TIBA (10^{-4}M)

Data: Fig. 41 (Table XXXVIII)

The two higher concentrations of TIBA induced 75% - 90% inhibition of the total uptake of IAA over 6 hours. TIBA at 10^{-5}M is apparently inhibitory for the first 2 hours only, after which a complete recovery sets in and uptake is subsequently of the same order as that of the control. During the first 2 hours, in the presence of the 2 higher concentrations of TIBA, the fluorescence values recorded for IAA were considerably higher than those of the control readings. A number of factors could possibly be responsible for this effect, including interference by compounds diffusing out of the tissue in the presence of high concentrations of TIBA in the external

FIG. 41


A IAA (10^{-5}M)
B IAA + TIBA (10^{-5}M)
C .. + .. (5×10^{-5}M)
D .. + .. (10^{-4}M)
5·0
4·0
3·0
2·0
1·0
0
−1·0
−2·0
MICROGRAMS IAA
1
2
3
4
5
6
TIME IN HOURS
A
B
C
D

medium, or merely experimental variation induced by the presence of some contaminant. This interference is evident until steady state uptake is attained.

With the erratic curves obtained when the uptake of IAA in the presence of high TIBA concentrations is plotted against time, the slopes, representing rate of uptake, calculated from either 1 - 6 hours or 0 - 6 hours, do not give a very true picture of the degree of inhibition. Table XXXVIII in the Appendix therefore includes the percentage inhibition by TIBA of the total uptake over 6 hours.

Experiment 2. A. IAA (2.5×10^{-5}M) Control

B. IAA (") + TIBA (10^{-5}M)

C. IAA (") + TIBA (2.5×10^{-5}M)

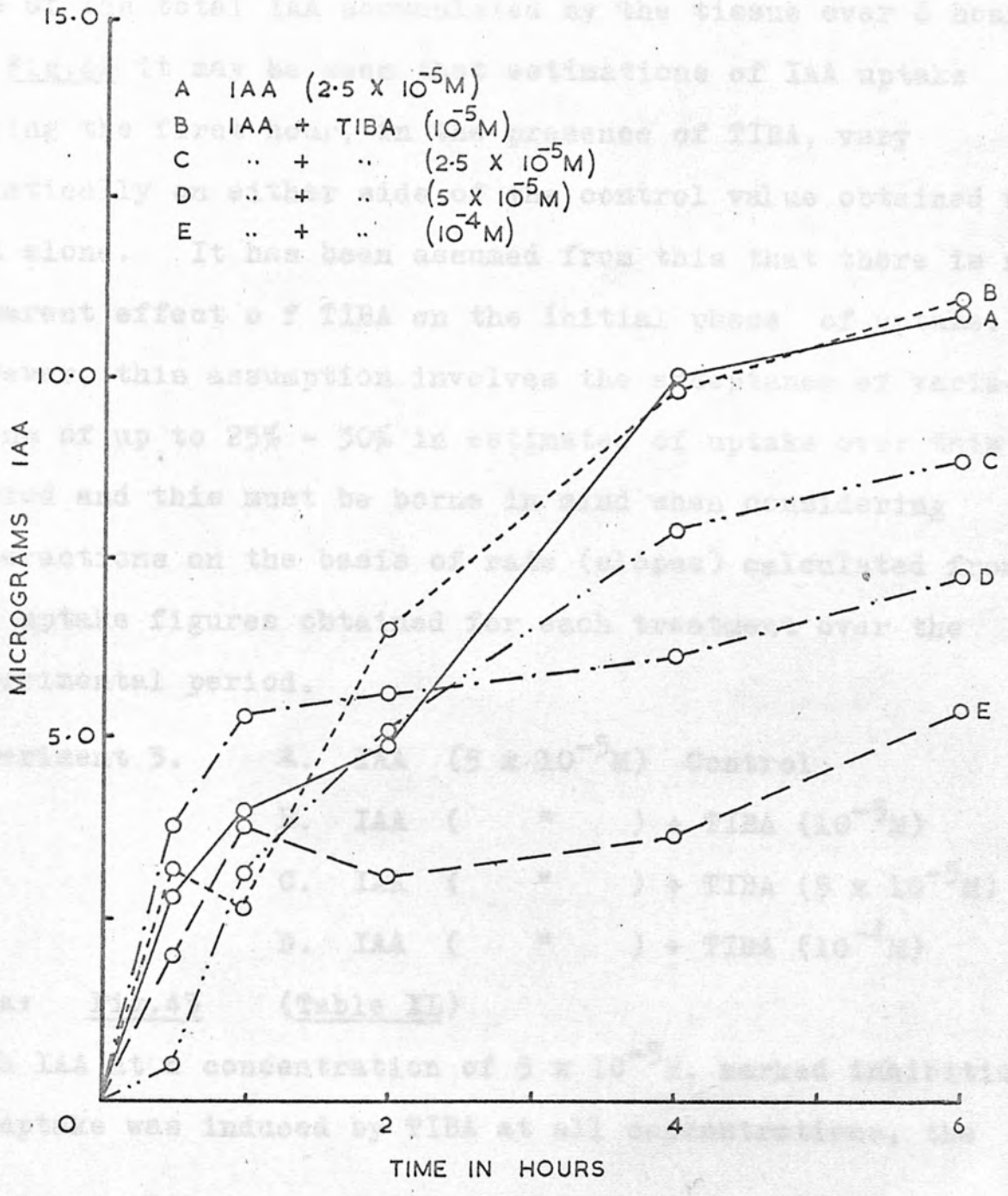
D. IAA (") + TIBA (5×10^{-5}M)

E. IAA (") + TIBA (10^{-4}M)

Data: Fig. 42 (Table XXXIX)

In this experiment there was no interference of the same magnitude as in the previous experiment with the fluorescence values obtained for the first two hours of sampling. The higher concentrations of TIBA were again inhibitory, whereas with a concentration of 10^{-5}M there was no significant effect on the uptake of IAA. The initial uptake of IAA over the first hour is much higher than the subsequent rate of metabolic uptake and is relatively unaffected by the

FIG. 42


15·0
10·0
5·0
0
2
4
6
MICROGRAMS IAA
TIME IN HOURS
A IAA (2·5 X 10⁻⁵M)
B IAA + TIBA (10⁻⁵M)
C .. + .. (2·5 X 10⁻⁵M)
D .. + .. (5 X 10⁻⁵M)
E .. + .. (10⁻⁴M)
B
A
C
D
E

presence of TIBA in the external solution. Depending on the degree of inhibition achieved by the series of TIBA concentrations, the initial phase of uptake accounts for 35% - 65% of the total IAA accumulated by the tissue over 6 hours. In Fig.42 it may be seen that estimations of IAA uptake during the first hour, in the presence of TIBA, vary erratically on either side of the control value obtained with IAA alone. It has been assumed from this that there is no apparent effect o f TIBA on the initial phase of uptake. However, this assumption involves the acceptance of variations of up to 25% - 30% in estimates of uptake over this period and this must be borne in mind when considering interactions on the basis of rate (slopes) calculated from the uptake figures obtained for each treatment over the experimental period.

Experiment 3. A. IAA (5×10^{-5}M) Control

B. IAA (") + TIBA (10^{-5}M)

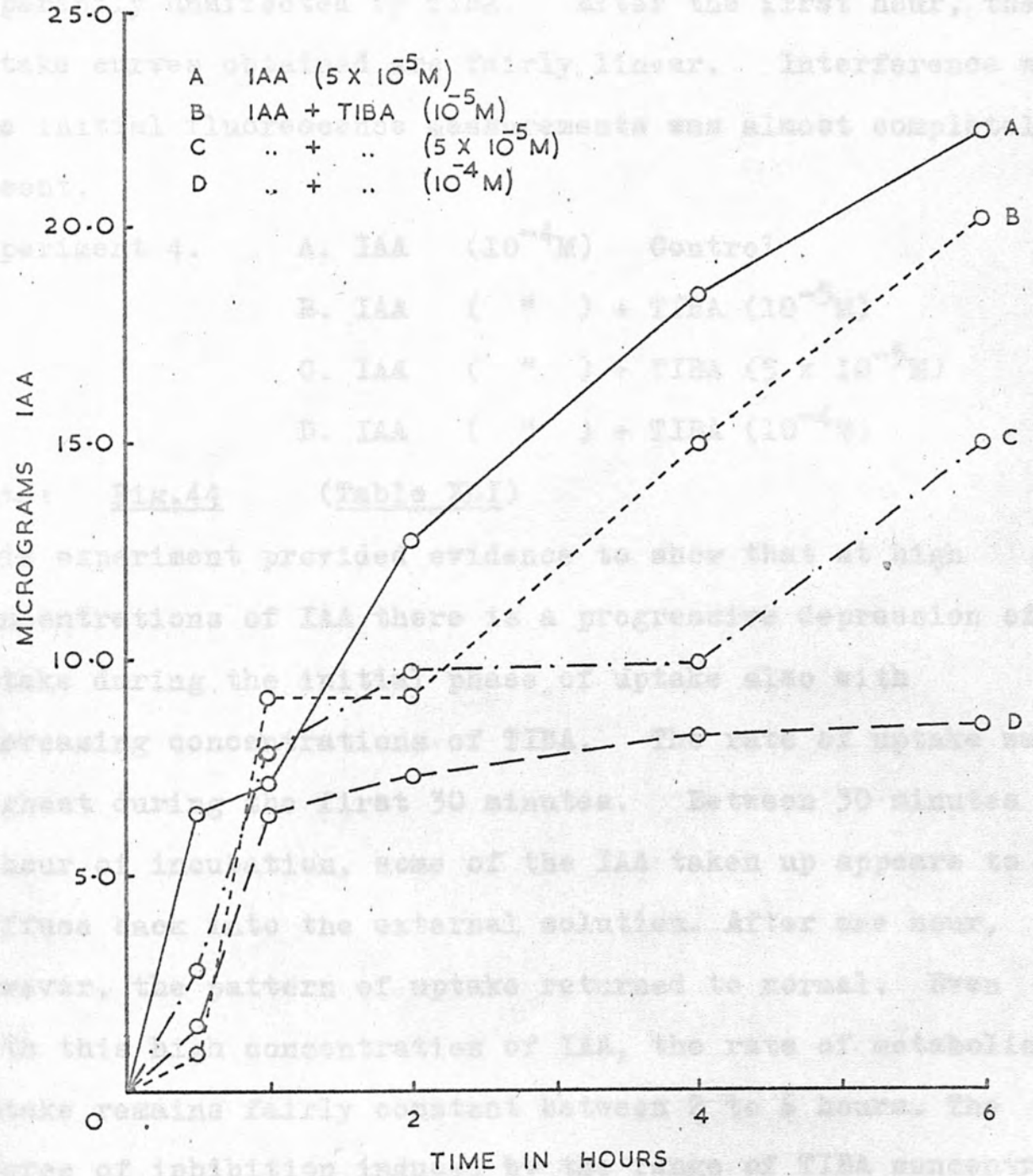
C. IAA (") + TIBA (5×10^{-5}M)

D. IAA (") + TIBA (10^{-4}M)

Data: Fig.43 (Table XL)

With IAA at a concentration of 5×10^{-5}M, marked inhibition of uptake was induced by TIBA at all concentrations, the

FIG. 43


A IAA (5 X 10⁻⁵M)
B IAA + TIBA (10⁻⁵M)
C .. + .. (5 X 10⁻⁵M)
D .. + .. (10⁻⁴M)
25·0
20·0
15·0
10·0
5·0
0
2
4
6
MICROGRAMS IAA
TIME IN HOURS
A
B
C
D

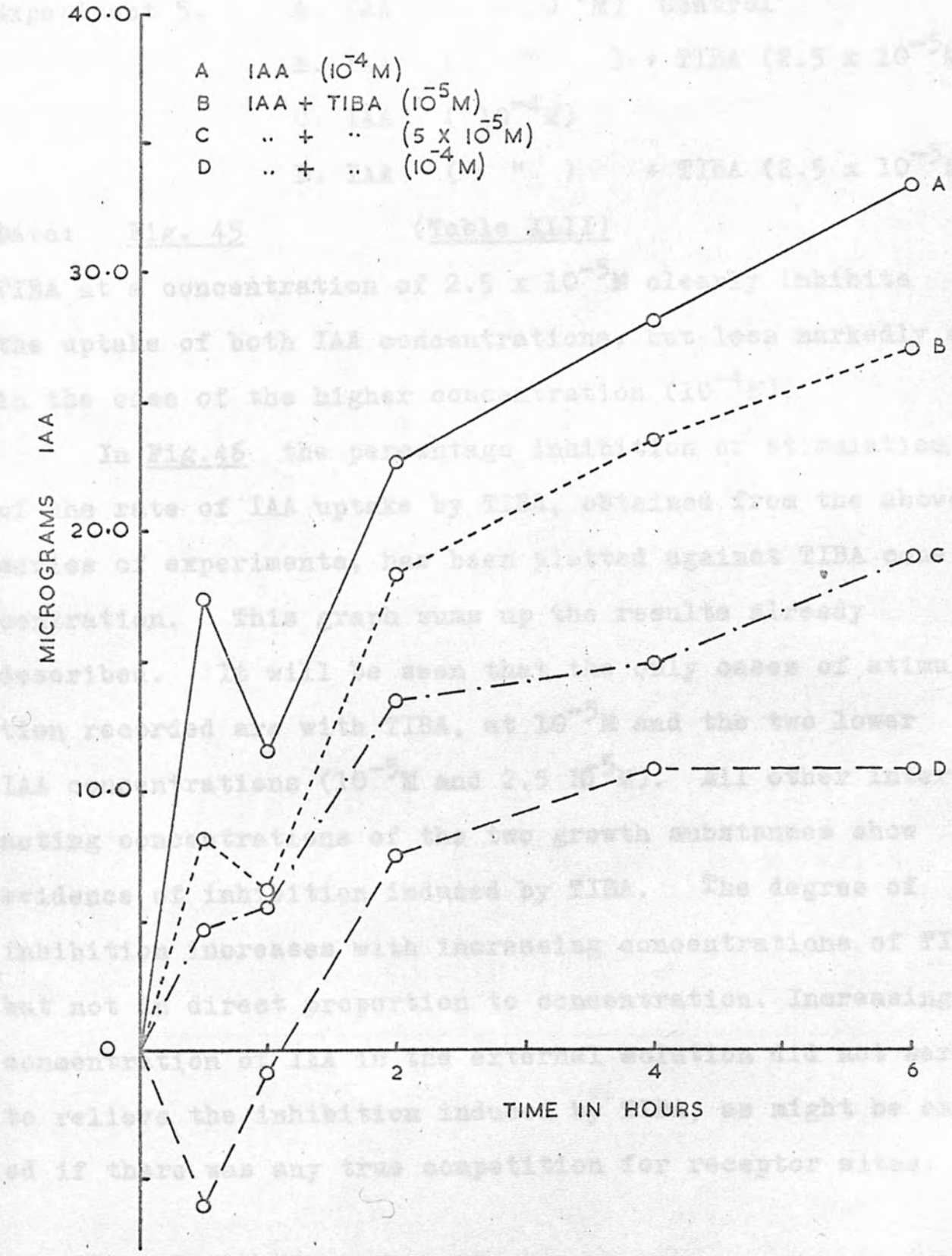
degree of inhibition increasing in a pronounced manner with increasing concentrations of TIBA. As was recorded in the previous experiment, the initial phase of uptake was apparently unaffected by TIBA. After the first hour, the uptake curves obtained are fairly linear. Interference with the initial fluorescence measurements was almost completely absent.

Experiment 4. A. IAA (10^{-4}M) Control
B. IAA (") + TIBA (10^{-5}M)
C. IAA (") + TIBA (5×10^{-5}M)
D. IAA (") + TIBA (10^{-4}M)

Data: Fig.44 (Table XLI)

This experiment provided evidence to show that at high concentrations of IAA there is a progressive depression of uptake during the initial phase of uptake also with increasing concentrations of TIBA. The rate of uptake was highest during the first 30 minutes. Between 30 minutes and 1 hour of incubation, some of the IAA taken up appears to diffuse back into the external solution. After one hour, however, the pattern of uptake returned to normal. Even with this high concentration of IAA, the rate of metabolic uptake remains fairly constant between 2 to 6 hours. The degree of inhibition induced by the range of TIBA concentrations appears to be somewhat reduced by the high concentration of IAA, indicating perhaps some form of recovery

FIG. 44


40·0
30·0
20·0
10·0
0
A IAA (10⁻⁴M)
B IAA + TIBA (10⁻⁵M)
C .. + .. (5 X 10⁻⁵M)
D .. + .. (10⁻⁴M)
A
B
C
D
MICROGRAMS IAA
2
4
6
TIME IN HOURS

due to competition for reactive sites of absorption or accumulation,

Experiment 5. A. IAA (5×10^{-5}M) Control

B. IAA (") + TIBA (2.5×10^{-5}M)

C. IAA (10^{-4}M)

D. IAA (") + TIBA (2.5×10^{-5}M)

Data: Fig. 45 (Table XLII)

TIBA at a concentration of 2.5×10^{-5}M clearly inhibits the uptake of both IAA concentrations, but less markedly so in the case of the higher concentration (10^{-4}M).

In Fig.46 the percentage inhibition or stimulation of the rate of IAA uptake by TIBA, obtained from the above series of experiments, has been plotted against TIBA concentration. This graph sums up the results already described. It will be seen that the only cases of stimulation recorded are with TIBA, at 10^{-5}M and the two lower IAA concentrations (10^{-5}M and 2.5×10^{-5}M). All other interacting concentrations of the two growth substances show evidence of inhibition induced by TIBA. The degree of inhibition increases with increasing concentrations of TIBA but not in direct proportion to concentration. Increasing the concentration of IAA in the external solution did not serve to relieve the inhibition induced by TIBA, as might be expected if there was any true competition for receptor sites.

FIG. 45

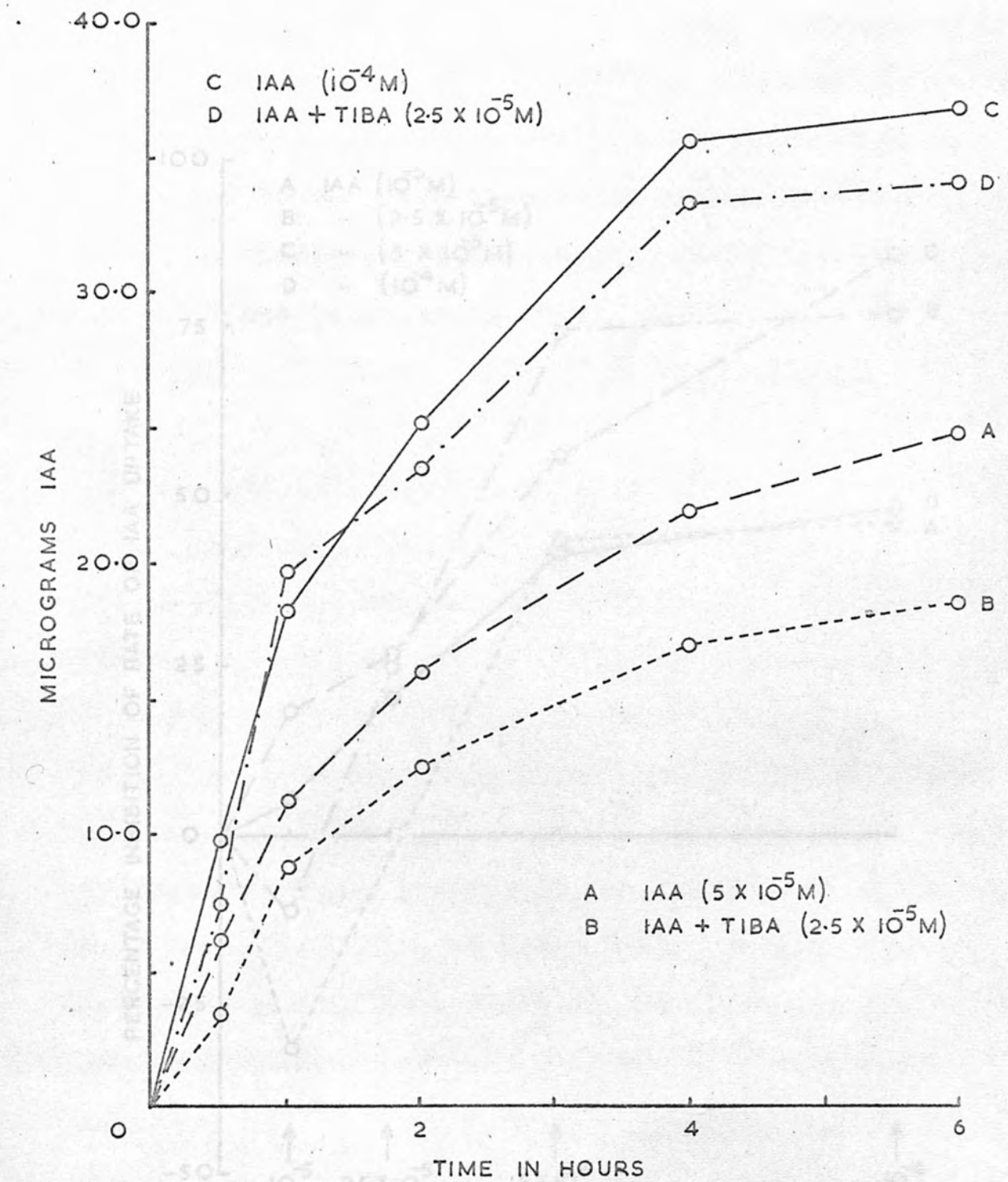
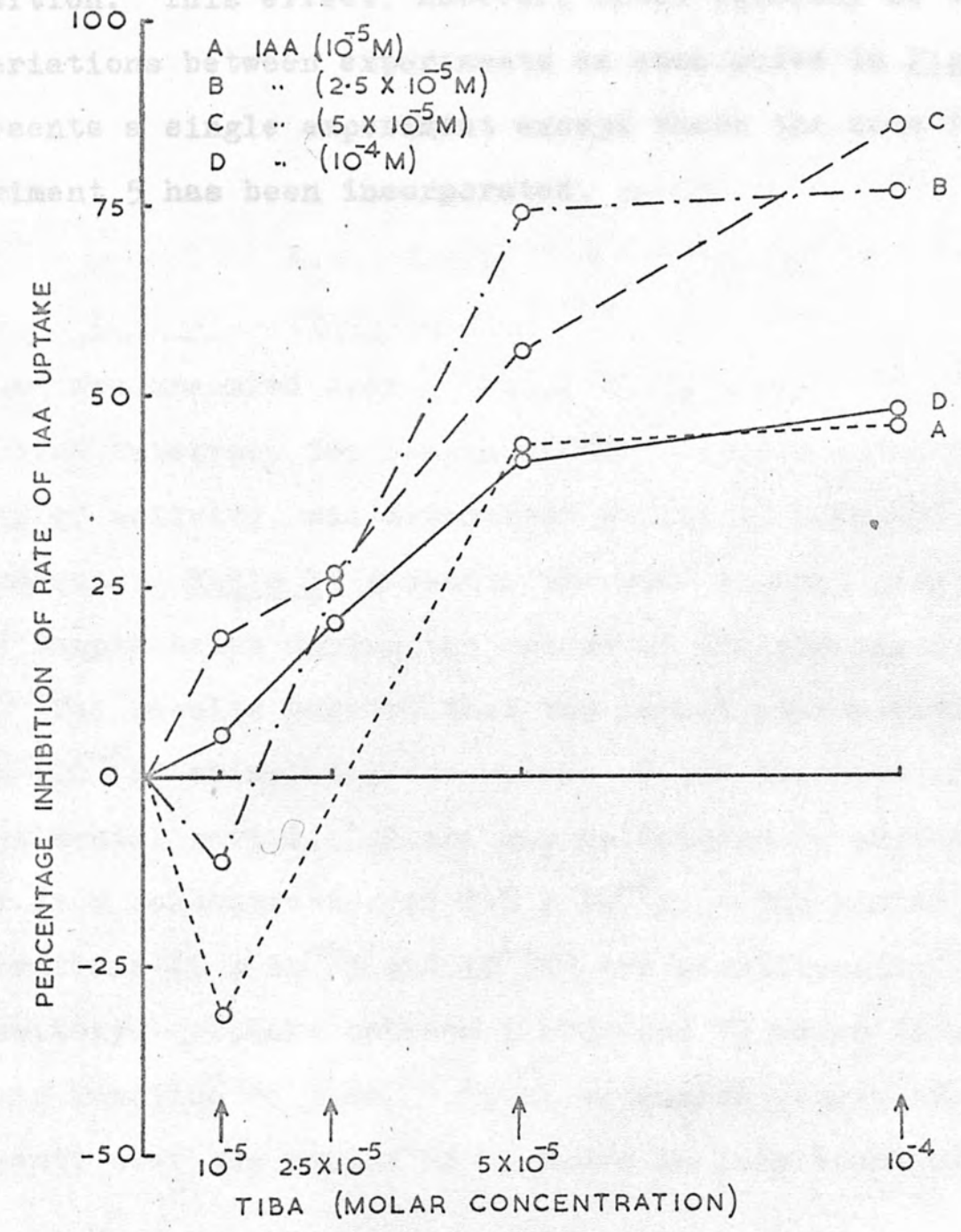

40·0
30·0
20·0
10·0
0
2
4
6
MICROGRAMS IAA
TIME IN HOURS
C IAA (10⁻⁴M)
D IAA + TIBA (2·5 X 10⁻⁵M)
A IAA (5 X 10⁻⁵M)
B IAA + TIBA (2·5 X 10⁻⁵M)
C
D
A
B

FIG. 46


A IAA (10^{-5}M)
B .. (2.5×10^{-5}M)
C .. (5×10^{-5}M)
D .. (10^{-4}M)
100
75
50
25
0
-25
-50
PERCENTAGE INHIBITION OF RATE OF IAA UPTAKE
C
B
D
A
10^{-5}
2.5×10^{-5}
5×10^{-5}
10^{-4}
TIBA (MOLAR CONCENTRATION)

On the contrary, increasing the IAA concentration appears to increase the degree of inhibition by TIBA which reaches a maximum at about 5 x 10^{-5}M IAA. Increasing the IAA concentration still further (10^{-4}M) seems to reduce this inhibition. This effect, however, could possibly be due to variations between experiments as each curve in Fig.46 represents a single experiment except where the data from Experiment 5 has been incorporated.

X (b)

MATERIAL: Zea mays - mesocotyl

METHOD: Radioactivity assay

GROWTH-SUBSTANCES: IAA - C^{14} + TIBA

Experiment 1.

A. IAA (10^{5}M) Control

B. IAA (") + TIBA (10^{-5}M)

C. IAA (") + TIBA (2.5×10^{-5}M)

D. IAA (") + TIBA (5×10^{-5}M)

E. IAA (") + TIBA (10^{-4}M)

Data: Fig. 47 (Table XLIII)

Uptake was measured over a period of 7½ hours. At each sampling interval, the length of the segments removed for assay of activity, was determined by the shadowgraph technique. Table 14 presents the mean segment length of each sample taken during the course of the experiment.

The results suggest that the lowest concentration of TIBA (10^{-5}M) stimulates the uptake of IAA over the entire experimental period. There was no detectable effect of TIBA at a concentration of 2.5×10^{-5}M. The higher concentrations (5×10^{-5}M and 10^{-4}M) are significantly inhibitory. Uptake between 1 hour and 7½ hours is a linear function of time. Total extension growth of the segments over the period of 7½ hours is less than 10% of

FIG. 47

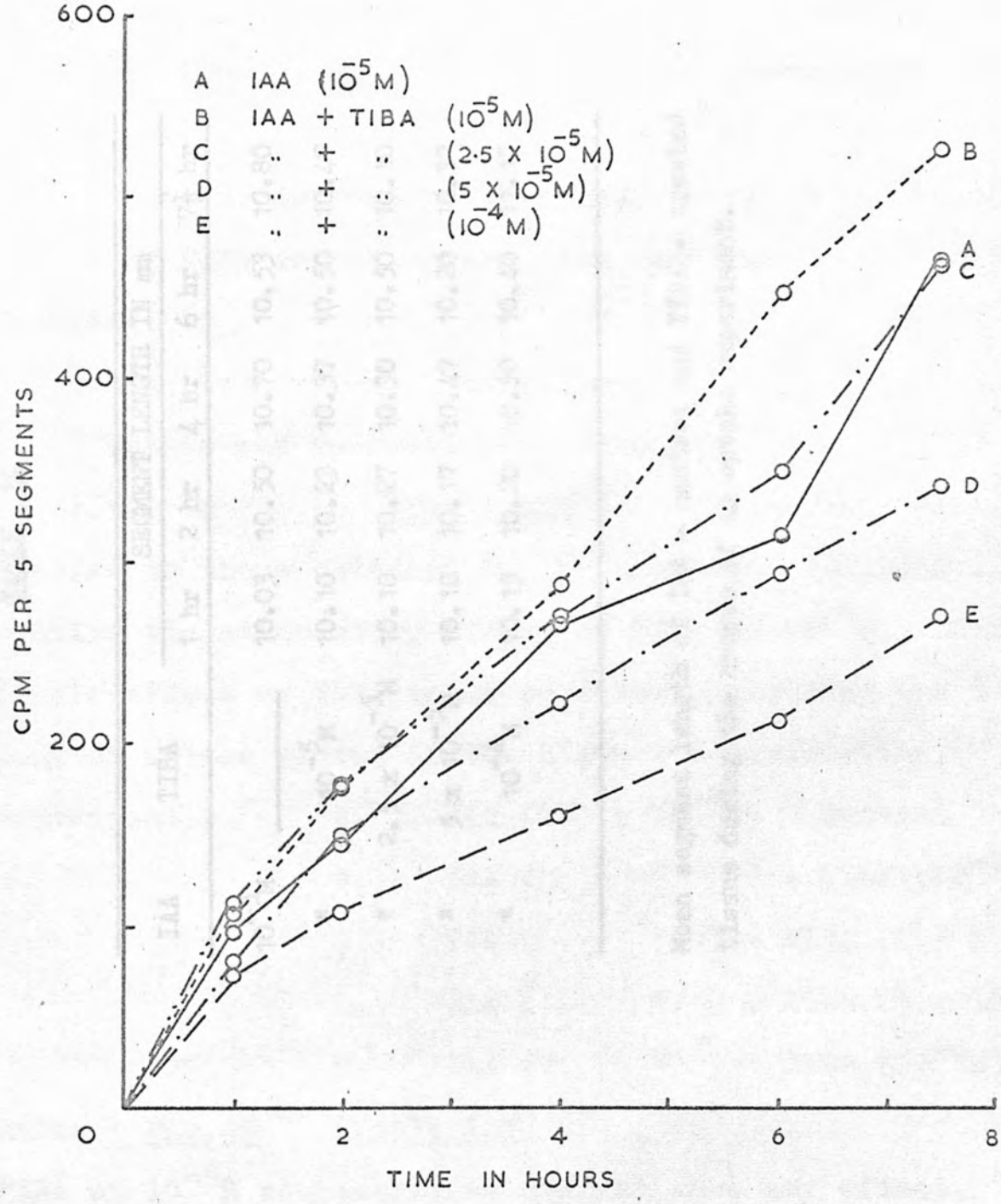

600
400
200
0
2
4
6
8
CPM PER 5 SEGMENTS
TIME IN HOURS
A IAA (10^{-5}M)
B IAA + TIBA (10^{-5}M)
C .. + .. (2.5×10^{-5}M)
D .. + .. (5×10^{-5}M)
E .. + .. (10^{-4}M)
A
B
C
D
E

TABLE 14

IAA	TIBA	SEGMENT LENGTH IN mm				
		1 hr	2 hr	4 hr	6 hr	$7\frac{1}{2}$ hr
10^{-5}M	_____	10.03	10.50	10.70	10.53	10.80
"	10^{-5}M	10.10	10.23	10.37	10.50	10.47
"	2.5×10^{-5}M	10.10	10.27	10.30	10.50	10.50
"	5×10^{-5}M	10.10	10.17	10.47	10.20	10.37
"	10^{-4}M	10.13	10.20	10.50	10.40	11.17

Mean segment length of IAA - control and TIBA - treated tissue during the course of an uptake experiment.

the initial segment length and is apparently unaffected by TIBA. The stimulation of uptake of the low IAA concentration (10^{-5}M) by low TIBA concentrations obtained in the above experiment agrees very well with the results obtained employing fluorescence assay and is of approximately the same magnitude.

In order to confirm this apparently synergistic effect of TIBA on IAA uptake, the experiment was carefully repeated.

Experiment 2: As Experiment 1.

Uptake was measured over 6 hours. The results are presented in Fig.48 (Table XLIV). The results were very similar to those obtained in the previous experiment, and confirm the stimulatory effect of TIBA at 10^{-5}M. Very little effect of TIBA could be discerned during the first hour of uptake except at the highest concentration.

Experiment 3.
A. IAA (2.5×10^{-5}M) Control
B. IAA (") + TIBA (10^{-5}M)
C. IAA (") + TIBA (2.5×10^{-5}M)
D. IAA (") + TIBA ($5.x\ 10^{-5}$M)
E. IAA (") + TIBA (10^{-4}M)

Data: Fig.49 (Table XLV)

TIBA at 10^{-5}M concentration did not show any effect,

stimulatory or inhibitory for the first 4 hours of uptake, after which some inhibition was indicated. All other concentrations were clearly inhibitory.

Experiment 4.
A. IAA (5×10^{-5}M) Control
B. IAA (") + TIBA (10^{-5}M)
C. IAA (") + TIBA (2.5×10^{-5}M)
D. IAA (") + TIBA (5×10^{-5}M)
E. IAA (") + TIBA (10^{-4}M)

Data: Fig.50 (Table XLVI)

In Table 15, the mean segment length of each sample assayed during the course of the experiments is given.

Experiment 5.
A. IAA (10^{-4}M)
B. IAA (") + TIBA (10^{-5}M)
C. IAA (") + TIBA (2.5×10^{-5}M)
D. IAA (") + TIBA (5×10^{-5}M)
E. IAA (") + TIBA (10^{-4}M)

Data: Fig.51 (Table XLVII)

In both the above experiments, TIBA at all concentrations was strongly inhibitory.

In Fig.52 the rates of uptake of IAA alone, and as affected by the series of TIBA concentrations, are plotted against the concentration of IAA. The degree of inhibition

FIG. 48

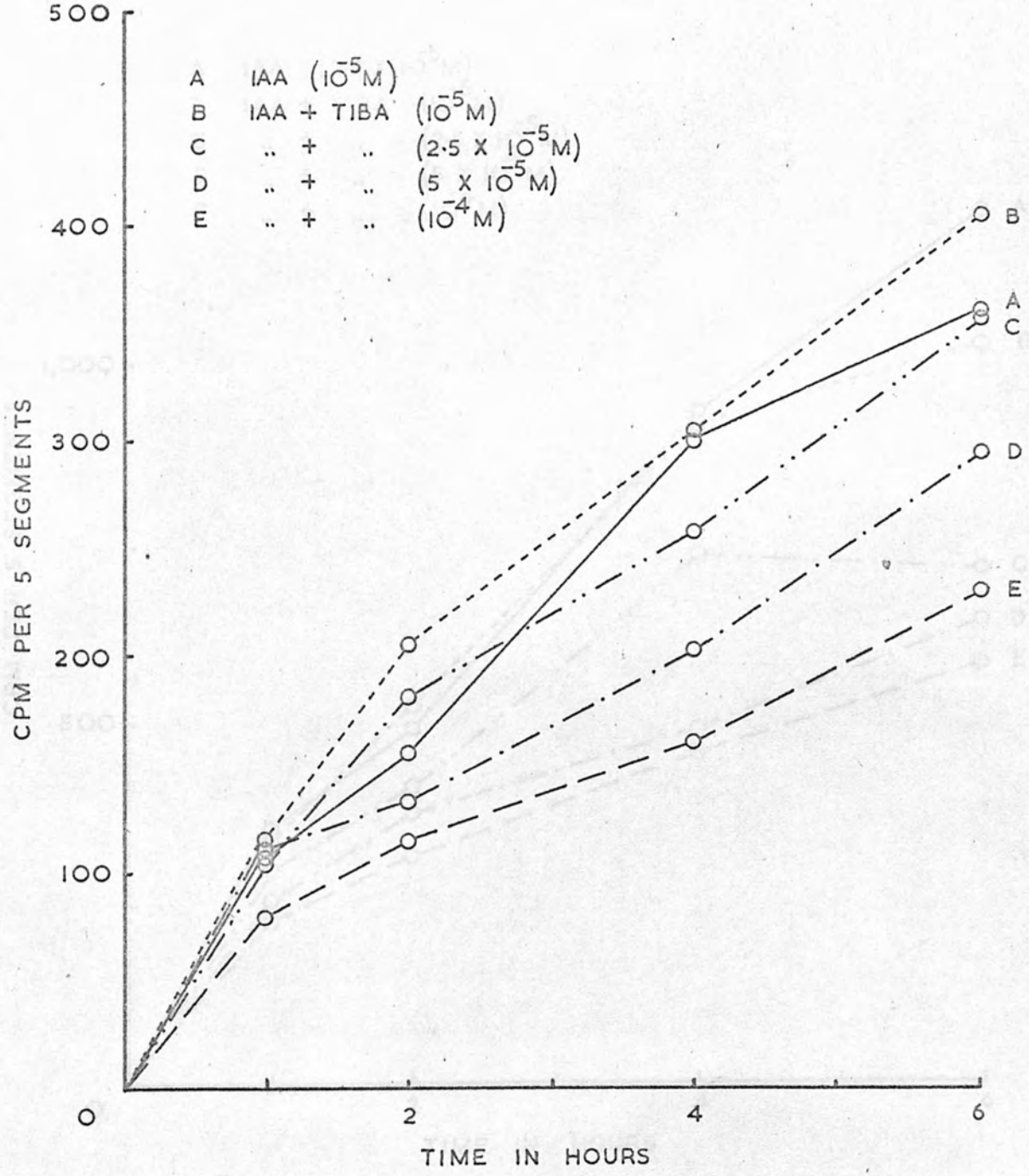

A IAA (10^{-5}M)
B IAA + TIBA (10^{-5}M)
C .. + .. (2.5×10^{-5}M)
D .. + .. (5×10^{-5}M)
E .. + .. (10^{-4}M)
CPM PER 5 SEGMENTS
TIME IN HOURS
0
100
200
300
400
500
2
4
6
A
B
C
D
E

FIG. 49

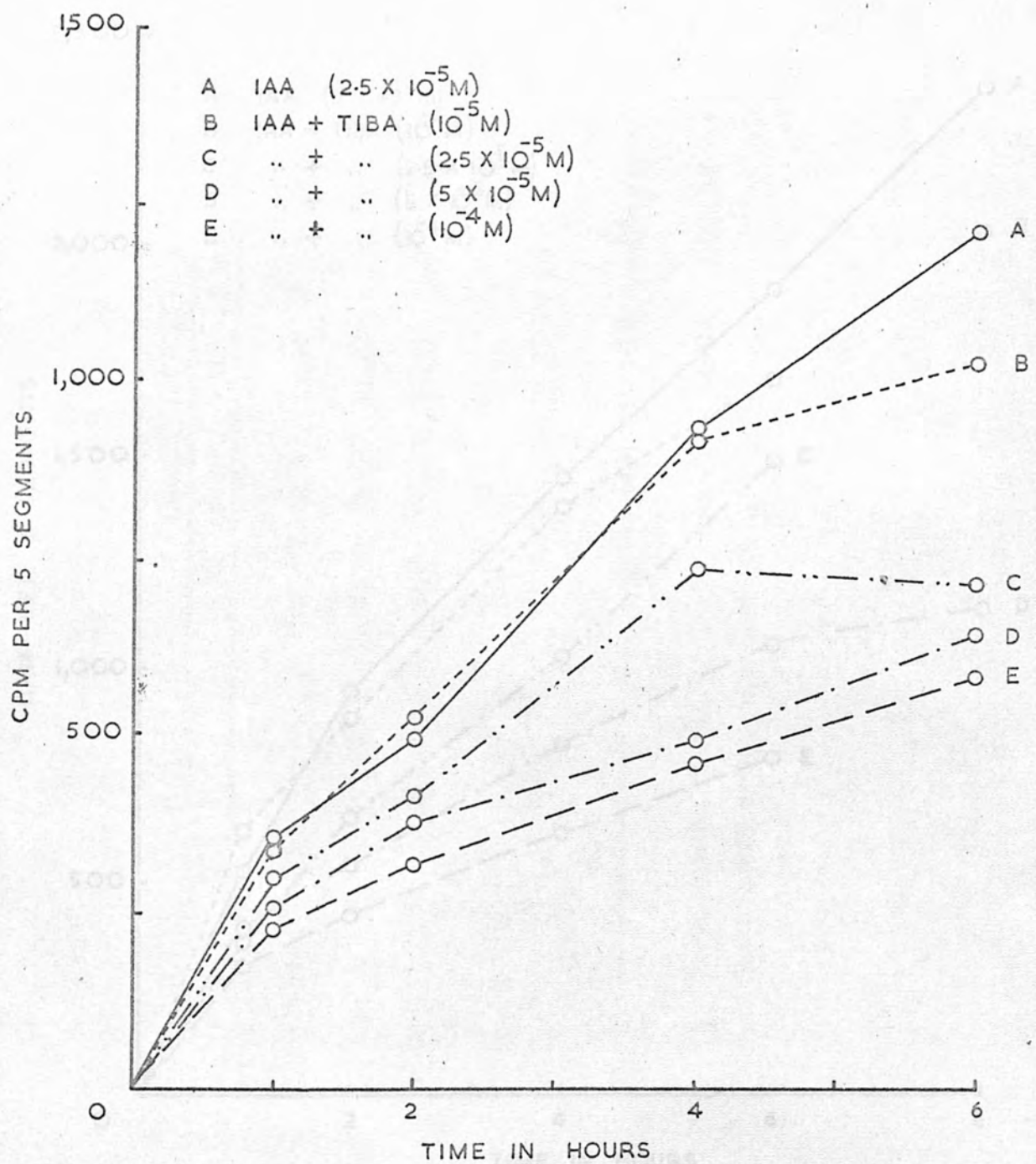

1,500
1,000
500
0
2
4
6
CPM PER 5 SEGMENTS
TIME IN HOURS
A IAA (2·5 X 10⁻⁵M)
B IAA + TIBA (10⁻⁵M)
C .. + .. (2·5 X 10⁻⁵M)
D .. + .. (5 X 10⁻⁵M)
E .. + .. (10⁻⁴M)
A
B
C
D
E

FIG. 50

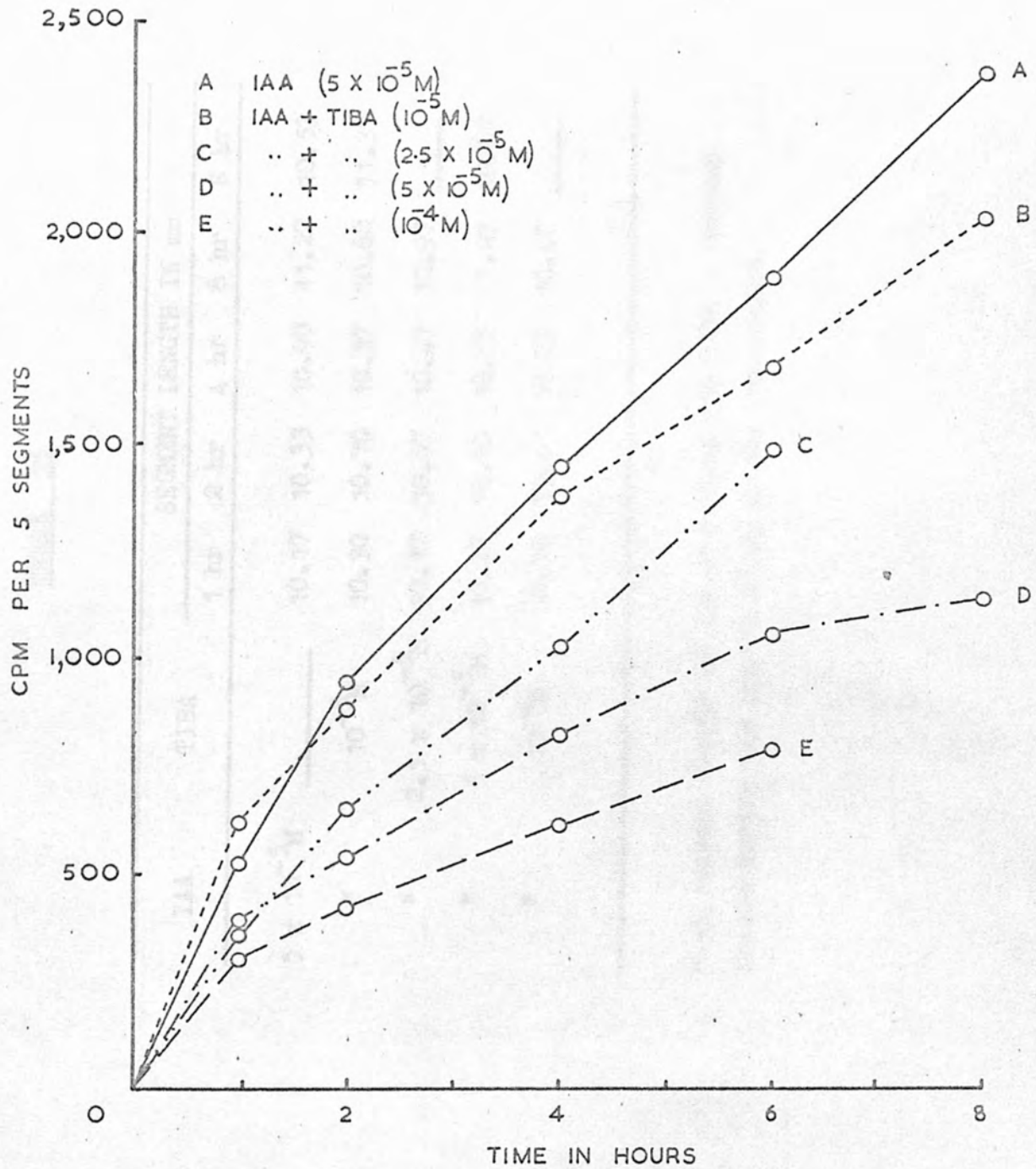
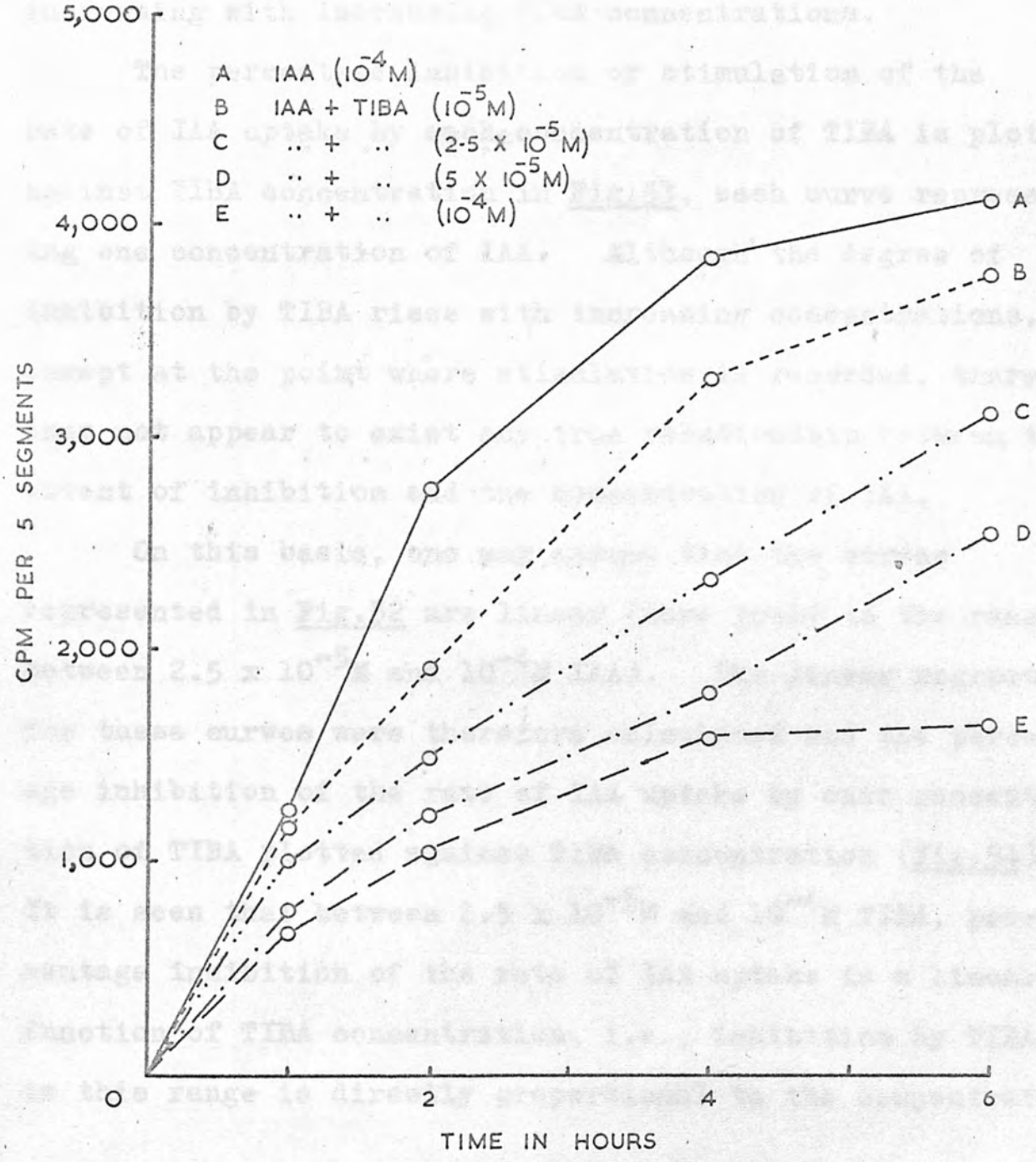

2,500
2,000
1,500
1,000
500
0
CPM PER 5 SEGMENTS
A IAA (5 X 10⁻⁵M)
B IAA + TIBA (10⁻⁵M)
C .. + .. (2·5 X 10⁻⁵M)
D .. + .. (5 X 10⁻⁵M)
E .. + .. (10⁻⁴M)
A
B
C
D
E
2
4
6
8
TIME IN HOURS

TABLE 15

IAA	TIBA	SEGMENT LENGTH IN mm				
		1 hr	2 hr	4 hr	6 hr	8 hr
5×10^{-5}M	_____	10.17	10.33	10.90	11.27	10.53
"	10^{-5}M	10.30	10.70	10.37	10.60	11.37
"	2.5×10^{-5}M	10.17	10.67	10.47	10.97	____
"	5×10^{-5}M	10.27	10.60	10.53	11.27	10.67
"	10^{-4}M	10.10	10.47	10.73	10.40	____

Mean segment length of IAA - control and TIBA - treated tissue during the course of an uptake experiment.

FIG. 51


5,000
4,000
3,000
2,000
1,000
0
2
4
6
CPM PER 5 SEGMENTS
TIME IN HOURS
A IAA (10^{-4}M)
B IAA + TIBA (10^{-5}M)
C .. + .. (2.5×10^{-5}M)
D .. + .. (5×10^{-5}M)
E .. + .. (10^{-4}M)
A
B
C
D
E

induced by each concentration of TIBA (except where stimulation is recorded at 10^{-5}M) appears to vary within limits for each IAA concentration, the extent of inhibition increasing with increasing TIBA concentrations.

The percentage inhibition or stimulation of the rate of IAA uptake by each concentration of TIBA is plotted against TIBA concentration in Fig.53, each curve representing one concentration of IAA. Although the degree of inhibition by TIBA rises with increasing concentrations, except at the point where stimulation is recorded, there does not appear to exist any true relationship between the extent of inhibition and the concentration of IAA.

On this basis, one may assume that the curves represented in Fig.52 are linear (more truly in the range between 2.5 x 10^{-5}M and 10^{-4}M IAA). The linear regressions for these curves were therefore calculated and the percentage inhibition of the rate of IAA uptake by each concentration of TIBA plotted against TIBA concentration (Fig.54). It is seen that between 2.5 x 10^{-5}M and 10^{-4}M TIBA, percentage inhibition of the rate of IAA uptake is a linear function of TIBA concentration, i.e., inhibition by TIBA in this range is directly proportional to the concentration.

FIG. 52

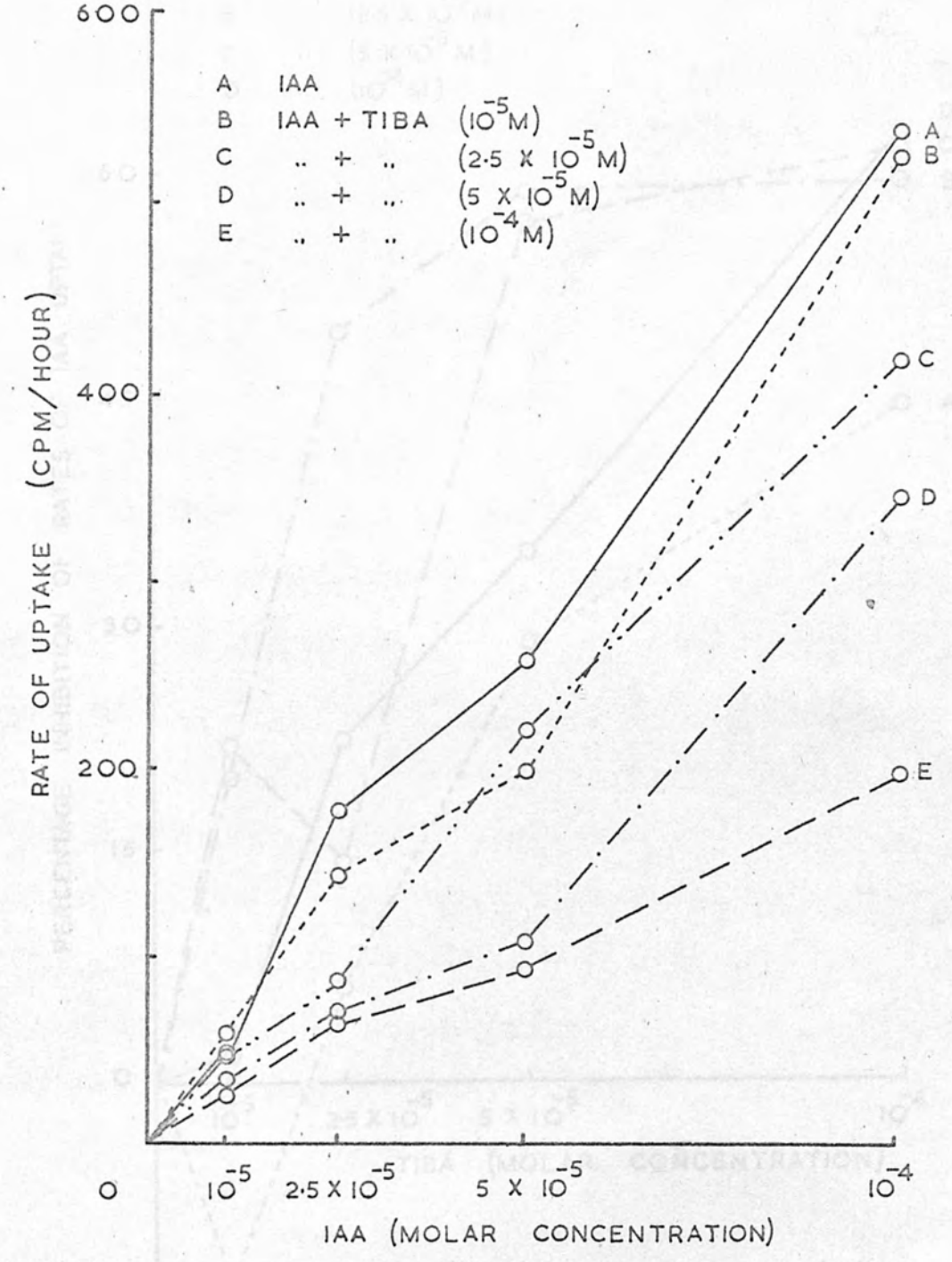

600
400
200
0
A IAA
B IAA + TIBA (10^{-5}M)
C .. + .. (2.5×10^{-5}M)
D .. + .. (5×10^{-5}M)
E .. + .. (10^{-4}M)
A
B
C
D
E
RATE OF UPTAKE (CPM/HOUR)
10^{-5}
2.5×10^{-5}
5×10^{-5}
10^{-4}
IAA (MOLAR CONCENTRATION)

FIG. 53

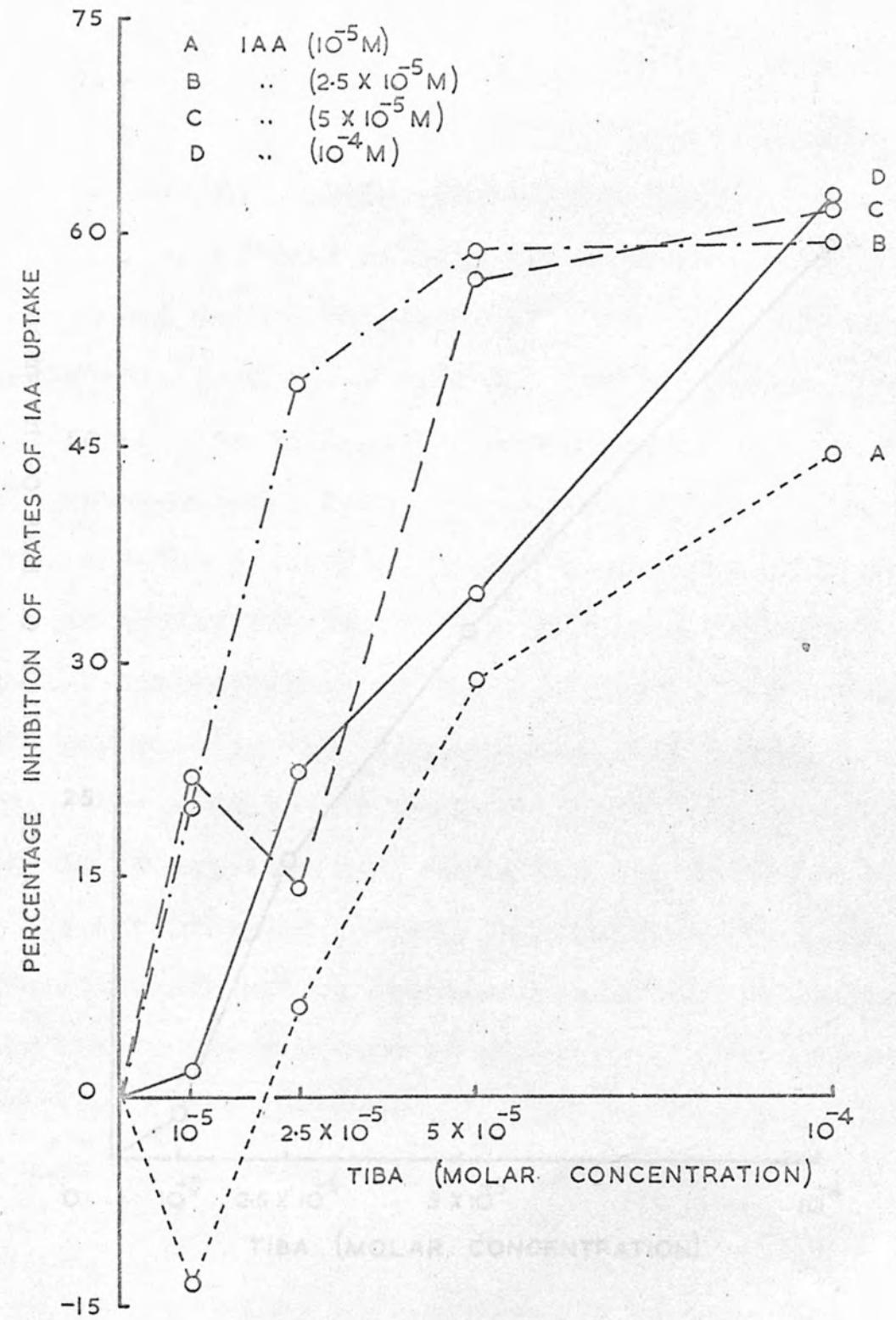
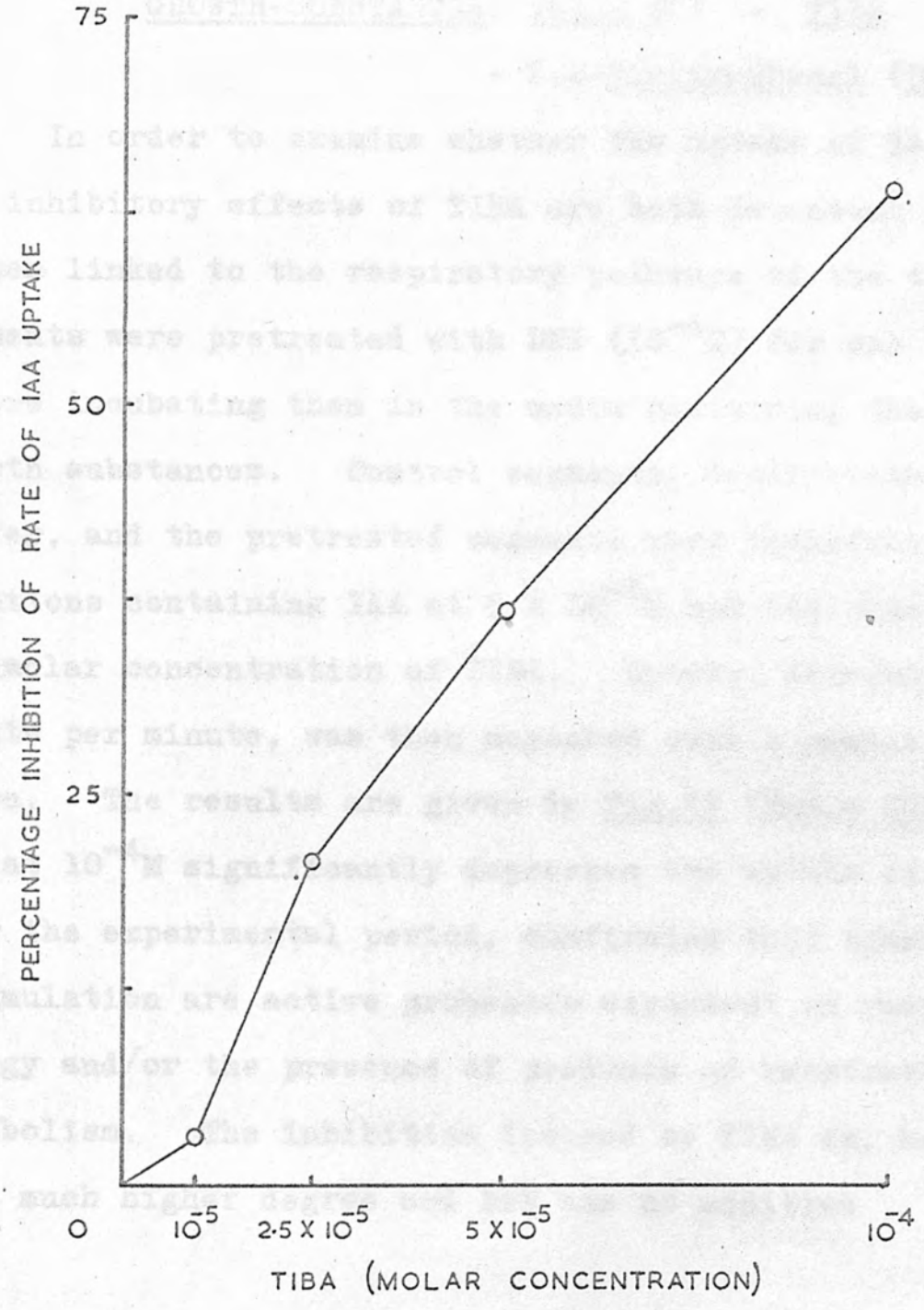

A IAA (10^{-5}M)
B .. (2.5×10^{-5}M)
C .. (5×10^{-5}M)
D .. (10^{-4}M)
PERCENTAGE INHIBITION OF RATES OF IAA UPTAKE
75
60
45
30
15
0
-15
10^{-5}
2.5×10^{-5}
5×10^{-5}
10^{-4}
TIBA (MOLAR CONCENTRATION)
D
C
B
A

FIG. 54


75
50
25
0
PERCENTAGE INHIBITION OF RATE OF IAA UPTAKE
10^{-5}
2.5×10^{-5}
5×10^{-5}
10^{-4}
TIBA (MOLAR CONCENTRATION)

XI

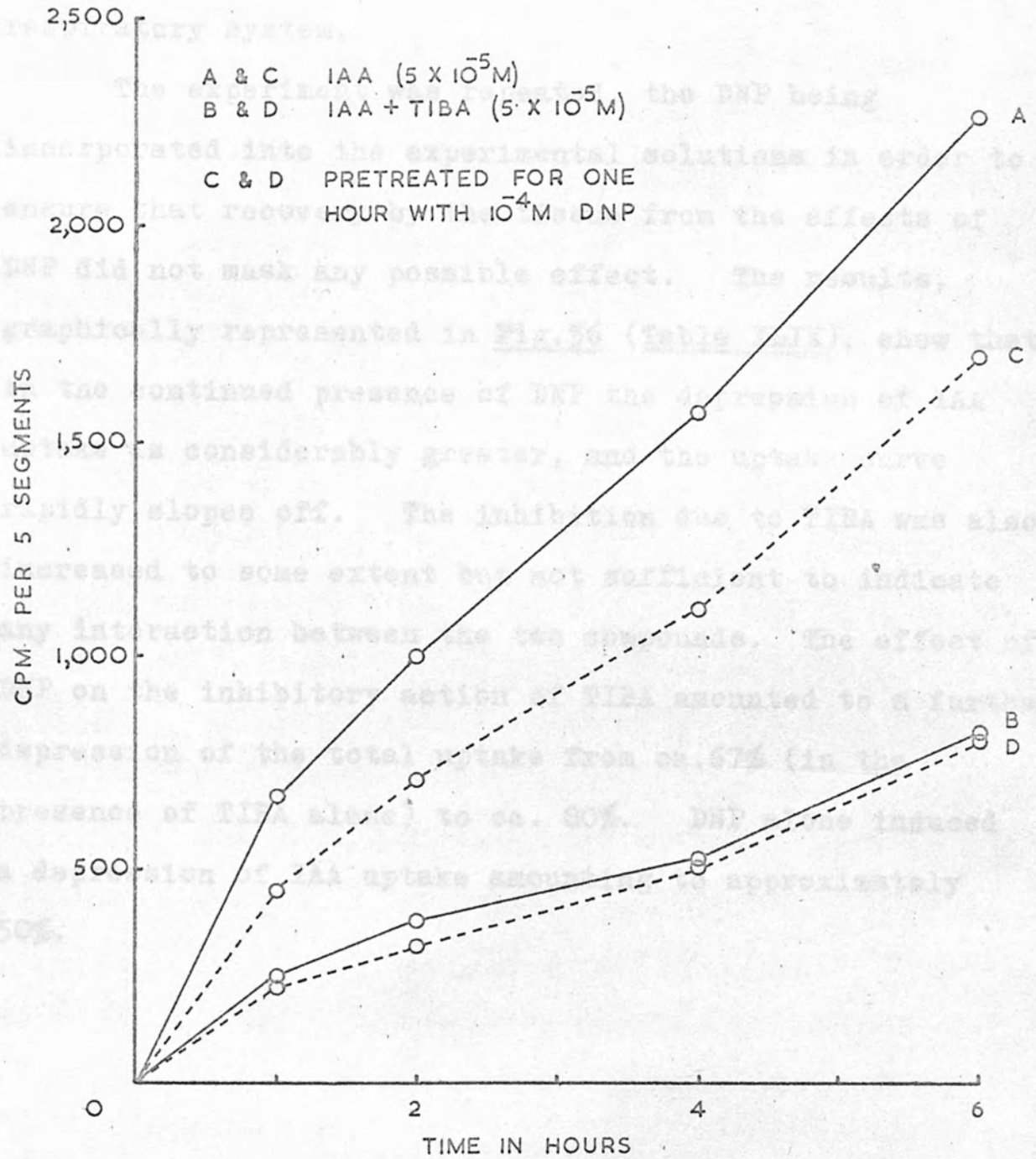
MATERIAL: Zea mays - mesocotyl

METHOD: Radioactivity assay

GROWTH-SUBSTANCES: IAA - C^{14} + TIBA + 2,4-Dinitrophenol (DNP)

In order to examine whether the uptake of IAA and the inhibitory effects of TIBA are both dependent on a system linked to the respiratory pathways of the tissue, segments were pretreated with DNP (10^{-4}M) for one hour before incubating them in the media containing the two growth substances. Control segments, equilibrated in buffer, and the pretreated segments were transferred to solutions containing IAA at 5×10^{-5}M and IAA plus an equimolar concentration of TIBA. Uptake, determined as counts per minute, was then measured over a period of 6 hours. The results are given in Fig.55 (Table XLVIII). DNP at 10^{-4}M significantly depresses the uptake of IAA over the experimental period, confirming that uptake and accumulation are active processes dependent on respiratory energy and/or the presence of products of respiratory metabolism. The inhibition induced by TIBA is, however, of a much higher degree and DNP has no additive

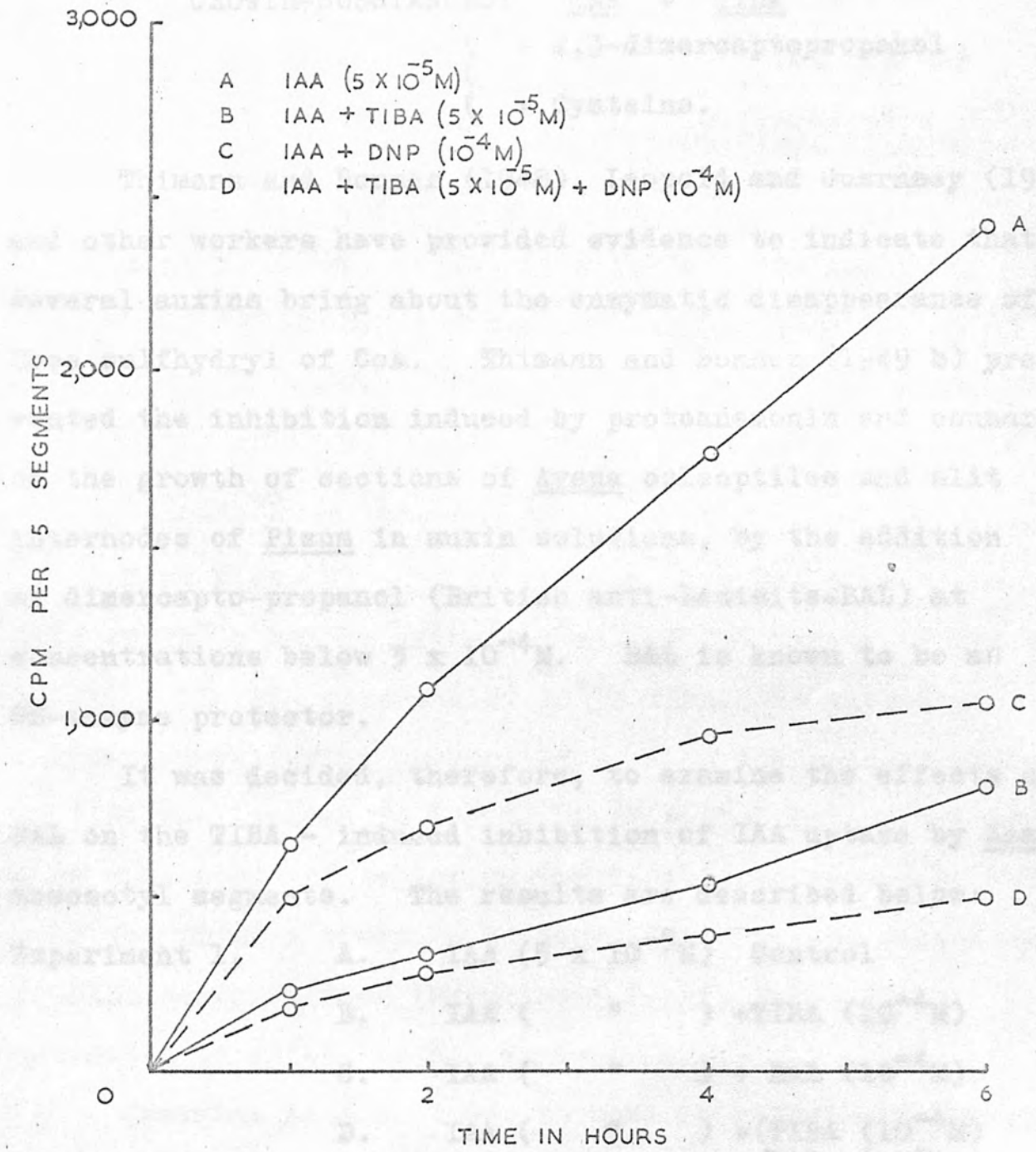
FIG. 55


2,500
2,000
1,500
1,000
500
0
CPM PER 5 SEGMENTS
A & C IAA (5 X 10⁻⁵M)
B & D IAA + TIBA (5 X 10⁻⁵M)
C & D PRETREATED FOR ONE HOUR WITH 10⁻⁴M DNP
A
C
B
D
2
4
6
TIME IN HOURS

multiplicative effect on this. It might be, therefore, that the inhibition evidenced by TIBA takes effect through a system unaffected by DNP and perhaps independent of the respiratory system.

The experiment was repeated, the DNP being incorporated into the experimental solutions in order to ensure that recovery by the tissue from the effects of DNP did not mask any possible effect. The results, graphically represented in Fig.56 (Table XLIX), show that in the continued presence of DNP the depression of IAA uptake is considerably greater, and the uptake curve rapidly slopes off. The inhibition due to TIBA was also increased to some extent but not sufficient to indicate any interaction between the two compounds. The effect of DNP on the inhibitory action of TIBA amounted to a further depression of the total uptake from ca.67% (in the presence of TIBA alone) to ca. 80%. DNP alone induced a depression of IAA uptake amounting to approximately 50%.

FIG. 56


3,000
2,000
1,000
0
2
4
6
CPM PER 5 SEGMENTS
TIME IN HOURS
A IAA (5 X 10⁻⁵M)
B IAA + TIBA (5 X 10⁻⁵M)
C IAA + DNP (10⁻⁴M)
D IAA + TIBA (5 X 10⁻⁵M) + DNP (10⁻⁴M)
A
B
C
D

XII

MATERIAL: Zea mays - mesocotyl

METHOD: Radioactivity assay

GROWTH-SUBSTANCES: IAA + TIBA
(+ 2,3-dimercaptopropanol
(+ Cysteine.

Thimann and Bonner (1948), Leopold and Guernsey (1953) and other workers have provided evidence to indicate that several auxins bring about the enzymatic disappearance of free sulfhydryl of CoA. Thimann and Bonner (1949 b) prevented the inhibition induced by protoanemonin and coumarin on the growth of sections of Avena coleoptiles and slit internodes of Pisum in auxin solutions, by the addition of dimercapto-propanol (British anti-Lewisite=BAL) at concentrations below 3×10^{-4}M. BAL is known to be an SH-enzyme protector.

It was decided, therefore, to examine the effects of BAL on the TIBA - induced inhibition of IAA uptake by Zea mesocotyl segments. The results are described below:

Experiment 1.
A. IAA (5×10^{-5}M) Control
B. IAA (") +TIBA (10^{-4}M)
C. IAA (") + BAL (10^{-4}M)
D. IAA (") +(TIBA (10^{-4}M)
(BAL (")

Uptake was measured over 6 hours.

Data: Fig.57 (Table L)

Experiment 2. A. IAA (5×10^{-5}M) Control

B. IAA (") + TIBA (5×10^{-5}M)

C. IAA (") + BAL (10^{-4}M)

D. IAA (") +(TIBA (5×10^{-5}M)
(BAL (10^{-4}M)

Data: Fig. 58 (Table LI)

Experiment 3. A. IAA (5×10^{-5}M) Control

B. IAA (") + TIBA (2.5×10^{-5}M)

C. IAA (") + BAL (10^{-4}M)

D. IAA (") +(TIBA (2.5×10^{-5}M)
(BAL (10-4M)

Data: Fig. 59 (Table LII)

From Figs. 57 - 59 it is seen that BAL at a concentration of 10^{-4}M has no effect at all on the uptake of IAA by itself. In the presence of 10^{-4}M TIBA, BAL showed no inhibition-relieving properties. When the concentration of TIBA was lowered to 5×10^{-5}M (Fig.58), BAL seemed to show some signs of relieving the inhibition caused by TIBA over the first 4 hours. However, lowering the concentration of TIBA still further (Experiment 3, Fig.59) again indicated no effect of BAL whatsoever.

Cysteine is also known to have SH-enzyme-protecting properties. An experiment was therefore conducted

FIG. 57

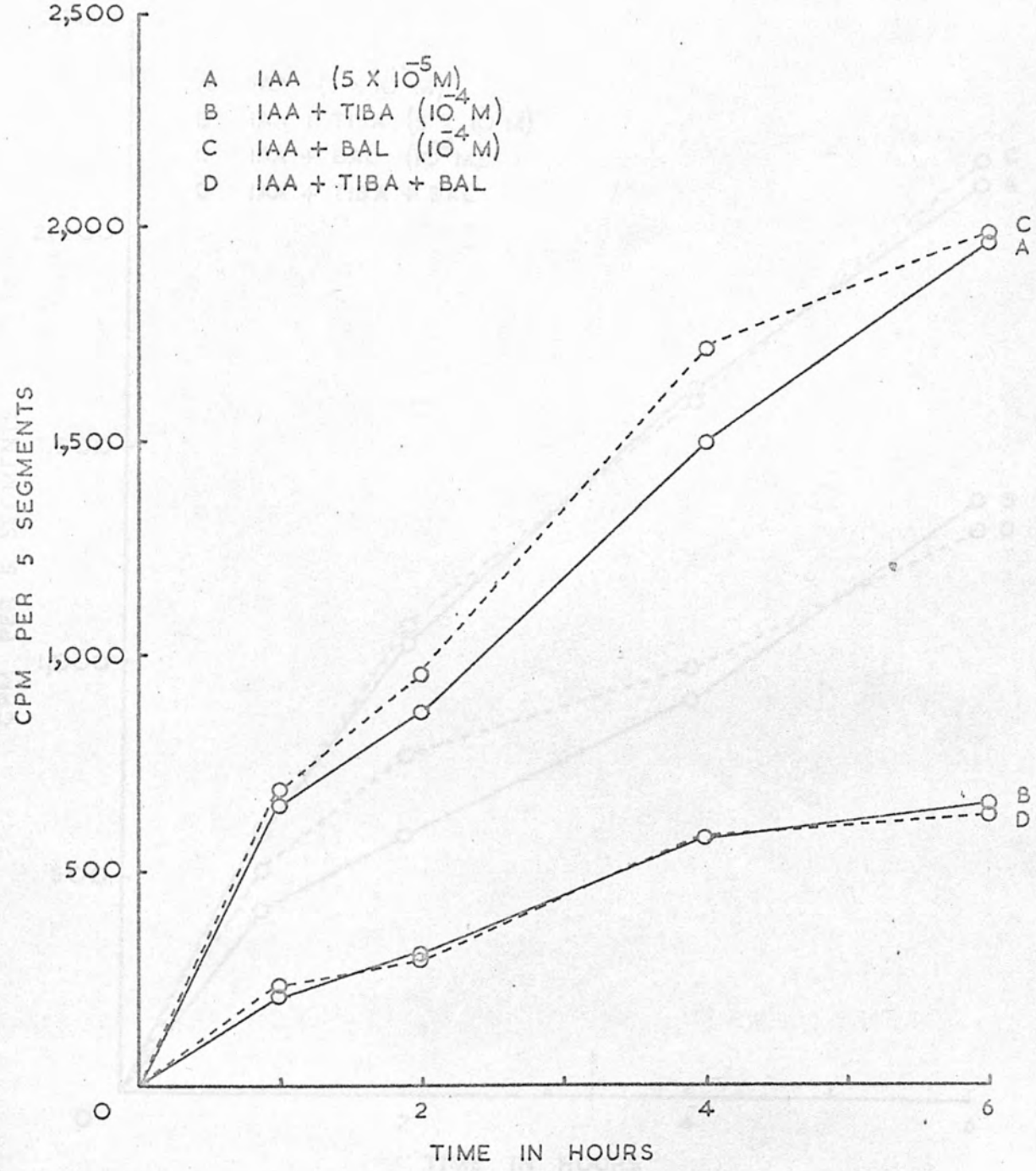

2,500
2,000
1,500
1,000
500
0
A IAA (5 X 10⁻⁵M)
B IAA + TIBA (10⁻⁴M)
C IAA + BAL (10⁻⁴M)
D IAA + TIBA + BAL
C
A
B
D
CPM PER 5 SEGMENTS
2
4
6
TIME IN HOURS

FIG. 58

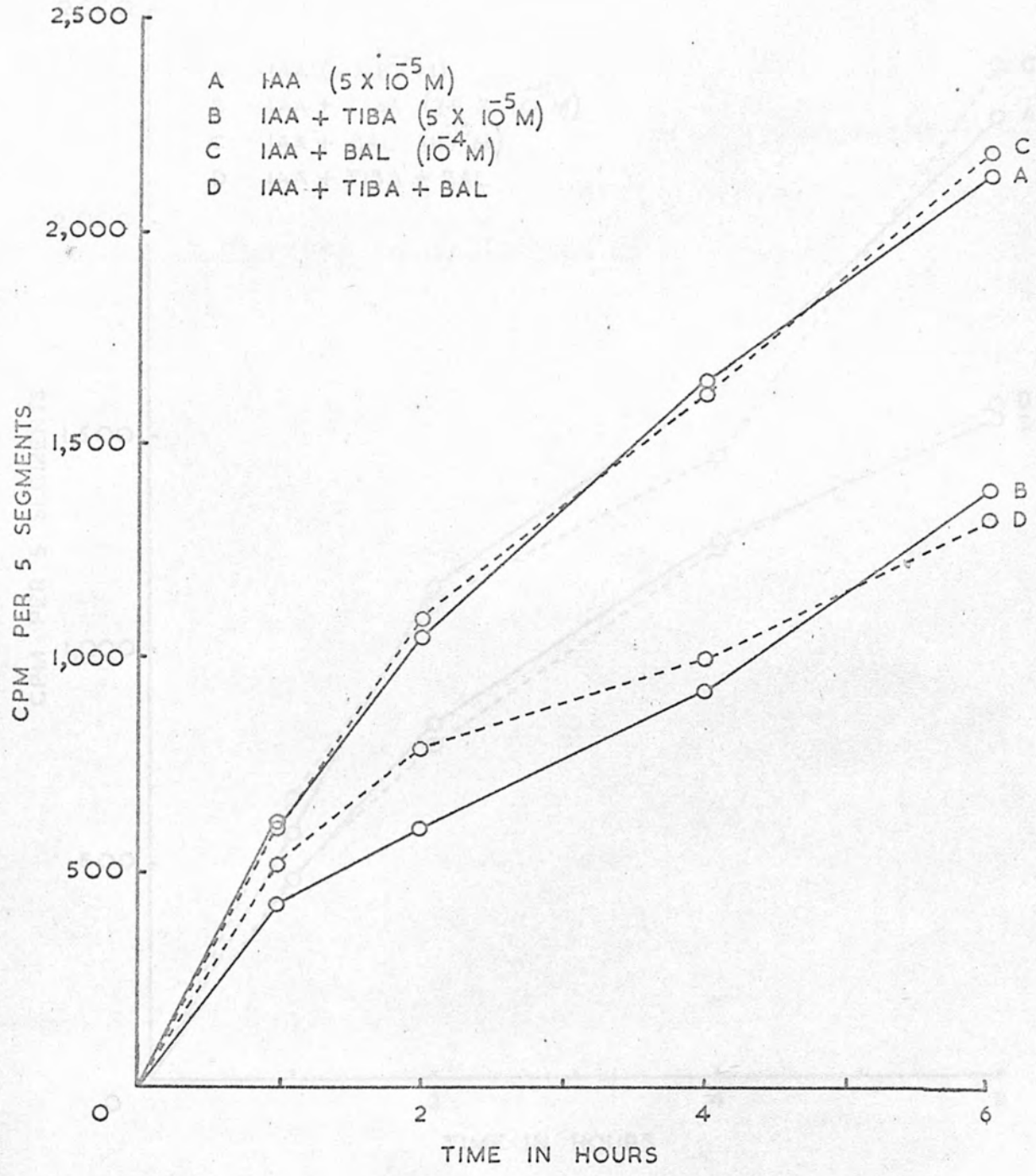
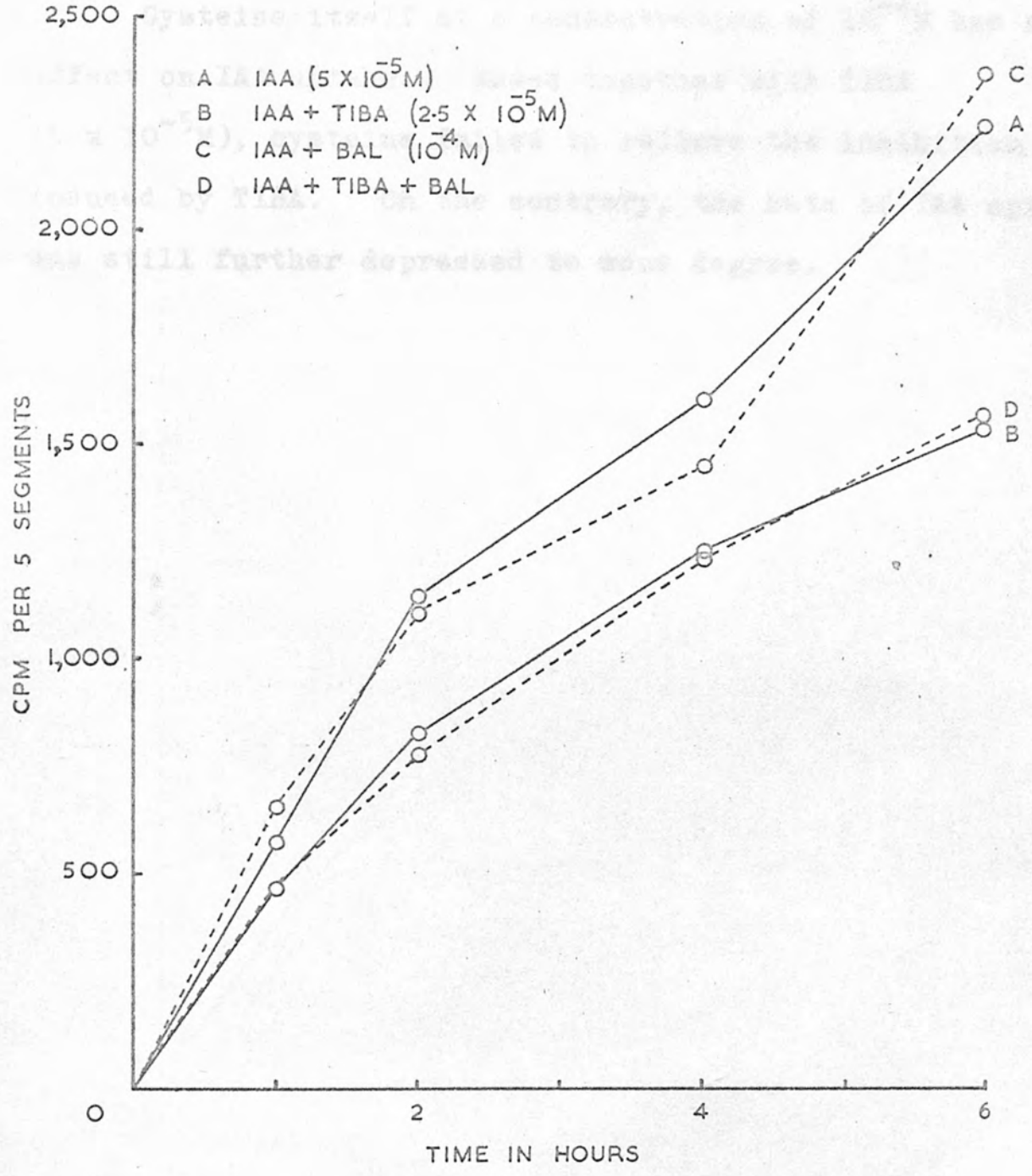

A IAA (5 X 10⁻⁵ M)
B IAA + TIBA (5 X 10⁻⁵ M)
C IAA + BAL (10⁻⁴ M)
D IAA + TIBA + BAL
2,500
2,000
1,500
1,000
500
0
CPM PER 5 SEGMENTS
2
4
6
TIME IN HOURS
C
A
B
D

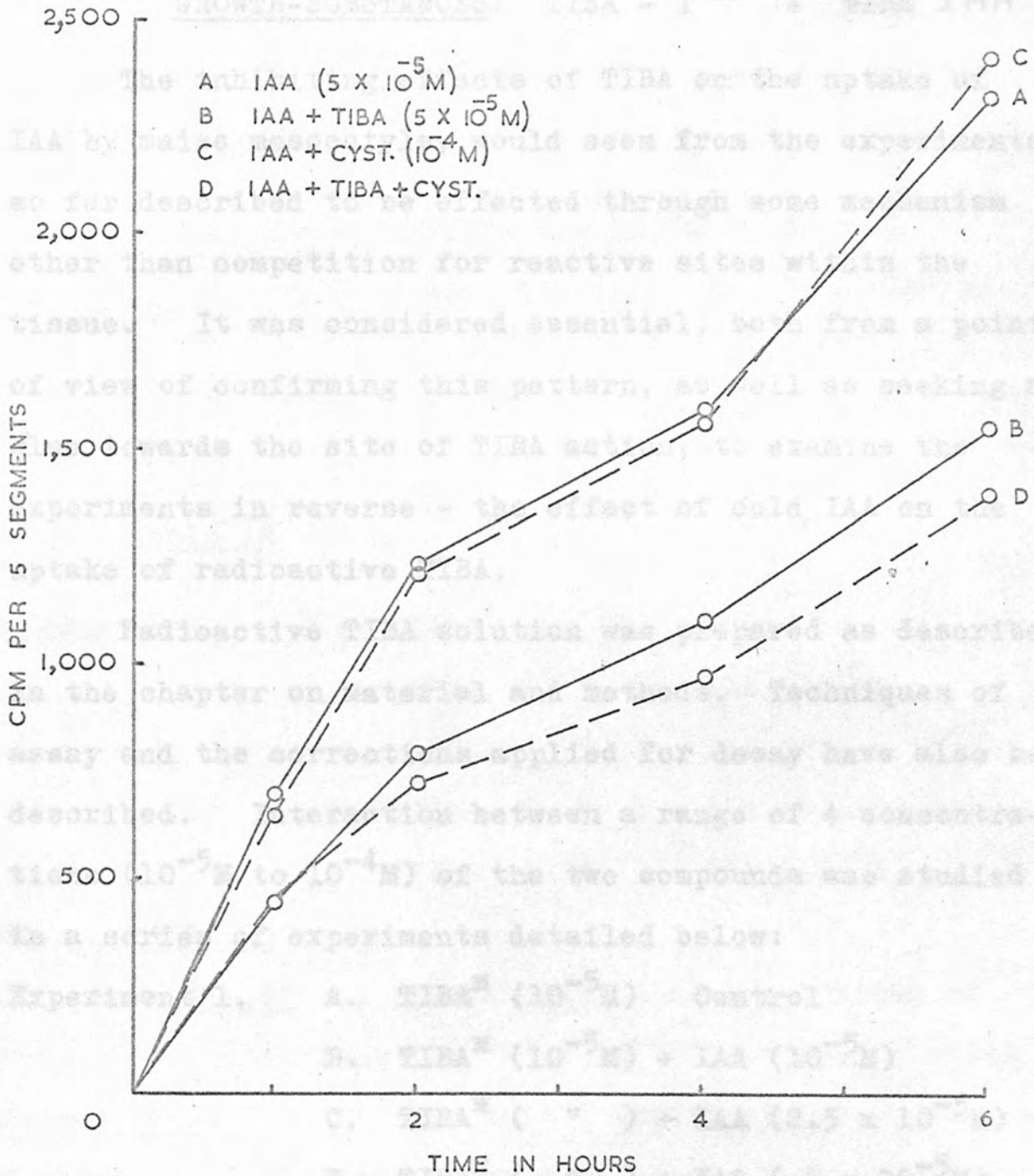
FIG. 59


2,500
2,000
1,500
1,000
500
0
CPM PER 5 SEGMENTS
A IAA (5 X 10⁻⁵M)
B IAA + TIBA (2·5 X 10⁻⁵M)
C IAA + BAL (10⁻⁴M)
D IAA + TIBA + BAL
C
A
D
B
2
4
6
TIME IN HOURS

employing this compound in order to confirm the results obtained with BAL. The data is presented in Fig.60 (Table LIII).

Cysteine itself at a concentration of 10^{-4}M has no effect on IAA uptake. Added together with TIBA (5×10^{-5}M), cysteine failed to relieve the inhibition induced by TIBA. On the contrary, the rate of IAA uptake was still further depressed to some degree.

FIG. 60


2,500
2,000
1,500
1,000
500
0
CPM PER 5 SEGMENTS
A IAA (5 X 10⁻⁵M)
B IAA + TIBA (5 X 10⁻⁵M)
C IAA + CYST. (10⁻⁴M)
D IAA + TIBA + CYST.
C
A
B
D
2
4
6
TIME IN HOURS

XIII

MATERIAL: Zea mays - mesocotyl

METHOD: Radioactivity assay

GROWTH-SUBSTANCES: TIBA - I^{131} + ~~TIBA~~ IAA

The inhibiting effects of TIBA on the uptake of IAA by maize mesocotyls, would seem from the experiments so far described to be effected through some mechanism other than competition for reactive sites within the tissue. It was considered essential, both from a point of view of confirming this pattern, as well as seeking a clue towards the site of TIBA action, to examine the experiments in reverse - the effect of cold IAA on the uptake of radioactive TIBA.

Radioactive TIBA solution was prepared as described in the chapter on material and methods. Techniques of assay and the corrections applied for decay have also been described. Interaction between a range of 4 concentrations (10^{-5}M to 10^{-4}M) of the two compounds was studied in a series of experiments detailed below:

Experiment 1.
- A. TIBA* (10^{-5}M) Control
- B. TIBA* (10^{-5}M) + IAA (10^{-5}M)
- C. TIBA* (") + IAA (2.5×10^{-5}M)
- D. TIBA* (") + IAA (5×10^{-5}M)
- E. TIBA* (") + IAA (10^{-4}M)

Data: Table 16

Experiment 2.

A. TIBA* (2.5×10^{-5}M) Control
B. TIBA (") + IAA (10^{-5}M)
C. TIBA (") + IAA (2.5×10^{-5}M)
D. TIBA (") + IAA (5×10^{-5}M)
E. TIBA (") + IAA (10^{-4}M)

Data: Table 17

Experiment 3.

A. TIBA* (5×10^{-5}M) Control
B. TIBA (") + IAA (10^{-5}M)
C. TIBA (") + IAA (2.5×10^{-5}M)
D. TIBA (") + IAA (5×10^{-5}M)
E. TIBA (") + IAA (10^{-4}M)

Data: Table 18

Experiment 4.

A. TIBA* (10^{-4}M) Control
B. TIBA (") + IAA (10^{-5}M)
C. TIBA (") + IAA (2.5×10^{-5}M)
D. TIBA (") + IAA (5×10^{-5}M)
E. TIBA (") + IAA (10^{-4}M)

Data: Table 19

In Fig.61 the uptake of TIBA from solutions of various concentrations (10^{-5}M to 10^{-4}M) has been plotted against time. It is seen that a family of smooth curves is obtained. The initial rate of uptake over the first hour is high, followed by a gradually declining rate of

TABLE 16

TIBA*	IAA	TIBA UPTAKE (CPM) 1 hr	2 hr	4 hr	6 hr	RATE
10^{-5}M	_____	2263	3823	5250	5552	875.5
"	10^{-5}M	2353	3683	4202	6038	880.9
"	2.5×10^{-5}M	2190	3554	4845	5524	858.9
"	5×10^{-5}M	2267	3939	4725	5479	829.9
"	10^{-4}M	2507	3282	5117	5540	862.9

Effect of 'cold' IAA on the uptake of radioactive TIBA by Zea mesocotyl segments.

Rate of uptake: - 0 __ 6 hours.

TABLE 17

TIBA*	IAA	TIBA UPTAKE (CPM)				RATE
		1 hr	2 hr	4 hr	6 hr	
2.5×10^{-5}M	_____	5616	9000	13,542	15,858	2521.1
"	10^{-5}M	5394	8916	12,606	15,774	2469.8
"	2.5×10^{-5}M	5712	9240	12,354	15,186	2338.1
"	5×10^{-5}M	6102	9348	14,094	16,050	2540.1
"	10^{-4}M	5466	9228	13,872	15,810	2538.4

Effect of 'cold' IAA on the uptake of radioactive TIBA by Zea mesocotyl segments.

Rate of uptake:- 0 __ 6 hours.

TABLE 18

TIBA*	IAA	TIBA UPTAKE (CPM)				RATE
		1 hr	2 hr	4 hr	6 hr	
5×10^{-5}M	_____	9522	15,030	25,128	32,664	5257.9
"	10^{-5}M	9504	15,882	22,536	29,664	4641.0
"	2.5×10^{-5}M	9564	14,676	21,432	32,208	4974.3
"	5×10^{-5}M	8832	14,640	24,492	30,732	4994.1
"	10^{-4}M	8250	13,386	23,148	28,356	4637.3

Effect of 'cold' IAA on the uptake of radioactive TIBA by Zea mesocotyl segments.

Rate of uptake:- 0 __ 6 hours.

TABLE 19

TIBA*	IAA	TIBA UPTAKE (CPM)				RATE
		1 hr	2 hr	4 hr	6 hr	
10^{-4}M	_____	19,219	31,149	46,574	55,665	8837.3
"	10^{-5}M	21,894	30,603	46,246	54,218	8435.0
"	2.5×10^{-5}M	19,874	29,784	46,628	57,002	9026.6
"	5×10^{-5}M	20,612	34,125	46,082	55,500	8610.4
"	10^{-4}M	21,130	29,484	46,219	60,197	9391.3

Effect of 'cold' IAA on the uptake of radioactive TIBA by Zea mesocotyl segments.
Rate of uptake:- 0 __ 6 hours.

uptake over time. The rate of uptake, calculated from 0 to 6 hours, is plotted in Fig. 62 as a function of TIBA concentration. A linear curve is thus obtained indicating that the uptake of TIBA over the concentration range studied, is directly proportional to its concentration in the external solution.

From the Tables (16 - 19) it is quite clear that IAA, at all concentrations tested, has no effect whatsoever on the entry and accumulation of TIBA.

FIG. 61: UPTAKE OF TIBA - I^{131}

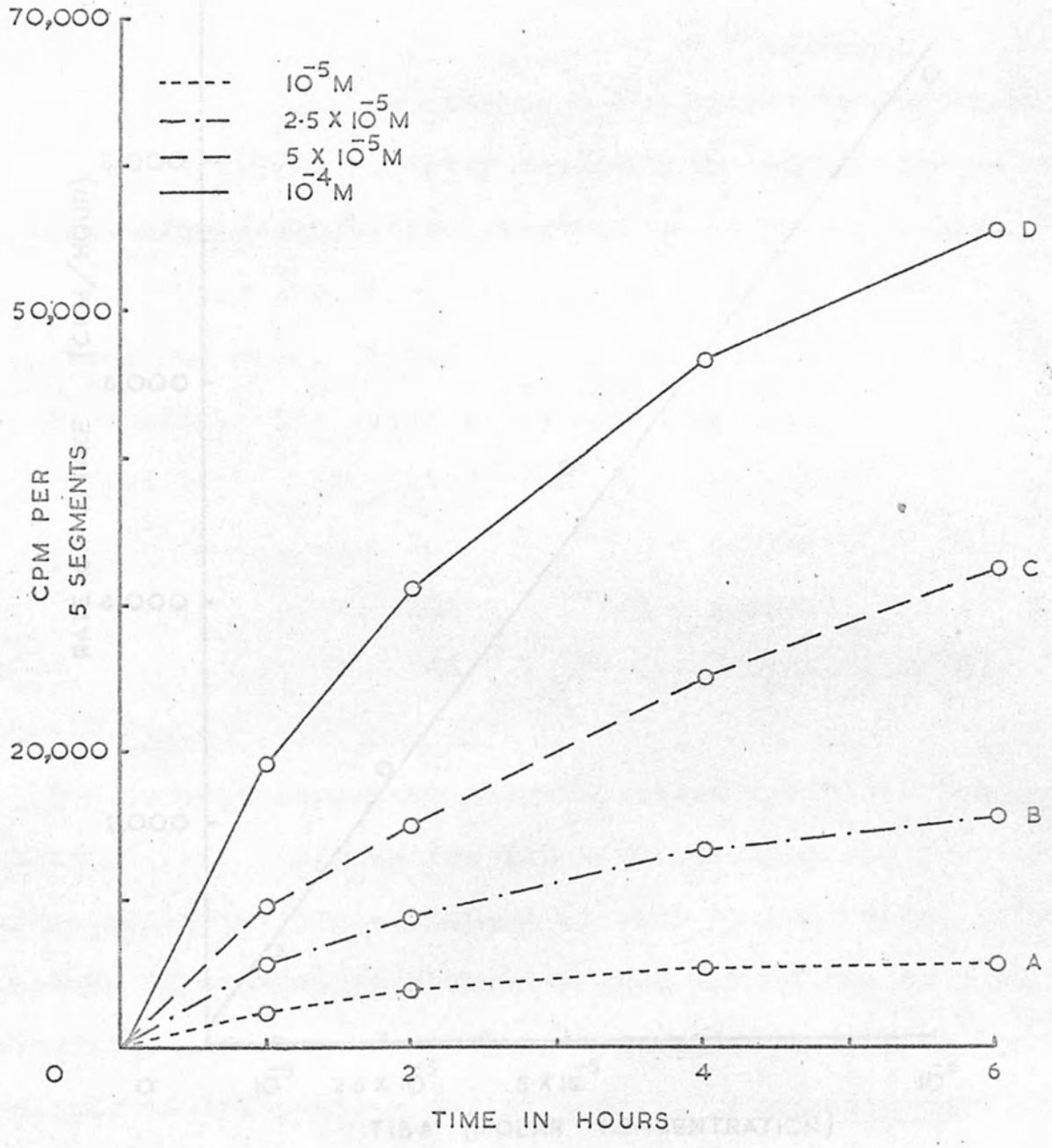
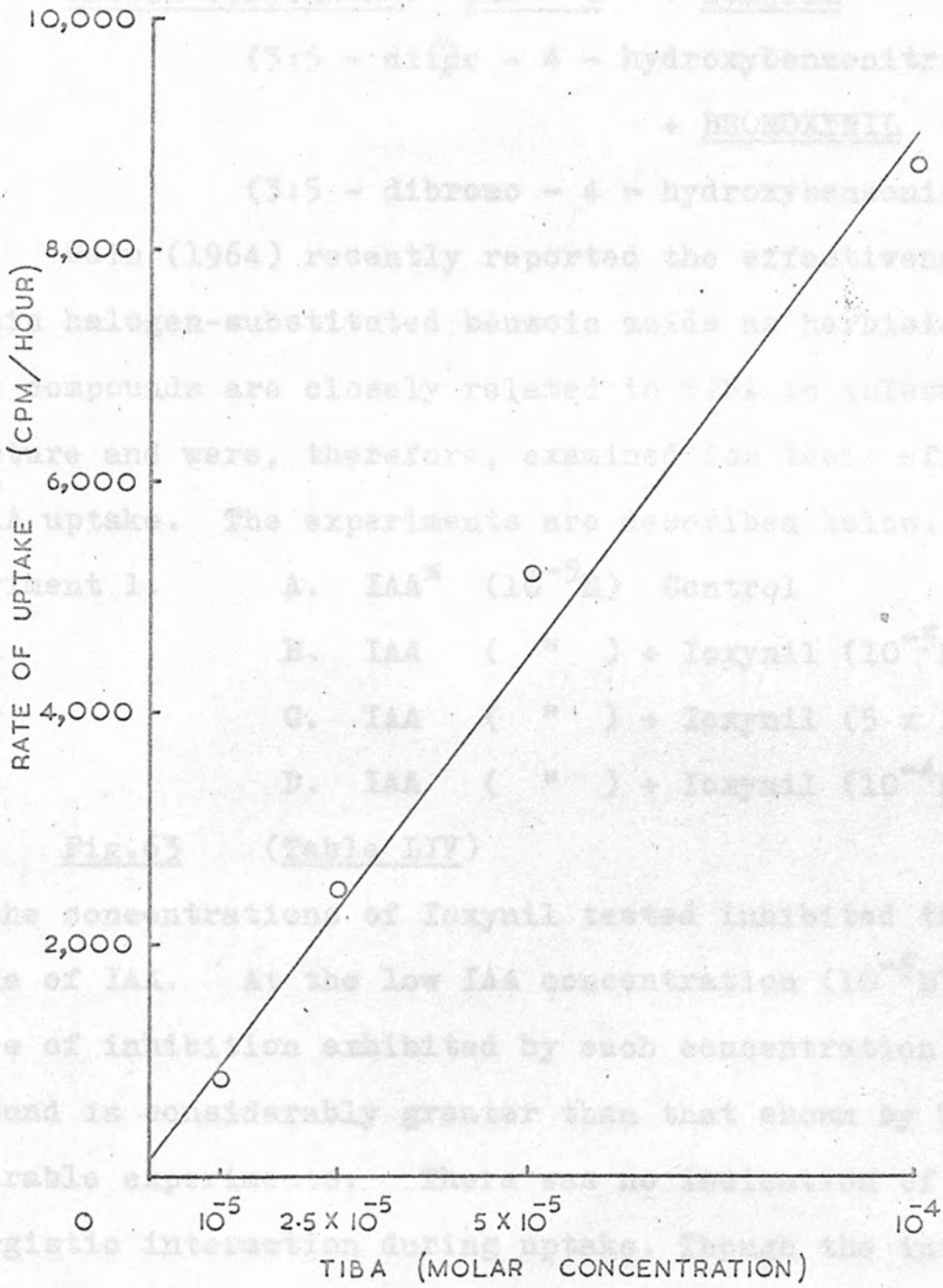


FIG. 62


10,000
8,000
6,000
4,000
2,000
0
RATE OF UPTAKE (CPM/HOUR)
10^{-5}
2.5 X 10^{-5}
5 X 10^{-5}
10^{-4}
TIBA (MOLAR CONCENTRATION)

XIV

MATERIAL: Zea mays - mesocotyl

METHOD: Radioactivity assay

GROWTH-SUBSTANCES: IAA - C^{14} + IOXYNIL -
(3:5 - diiodo - 4 - hydroxybenzonitrile)
+ BROMOXYNIL
(3:5 - dibromo - 4 - hydroxybenzonitrile)

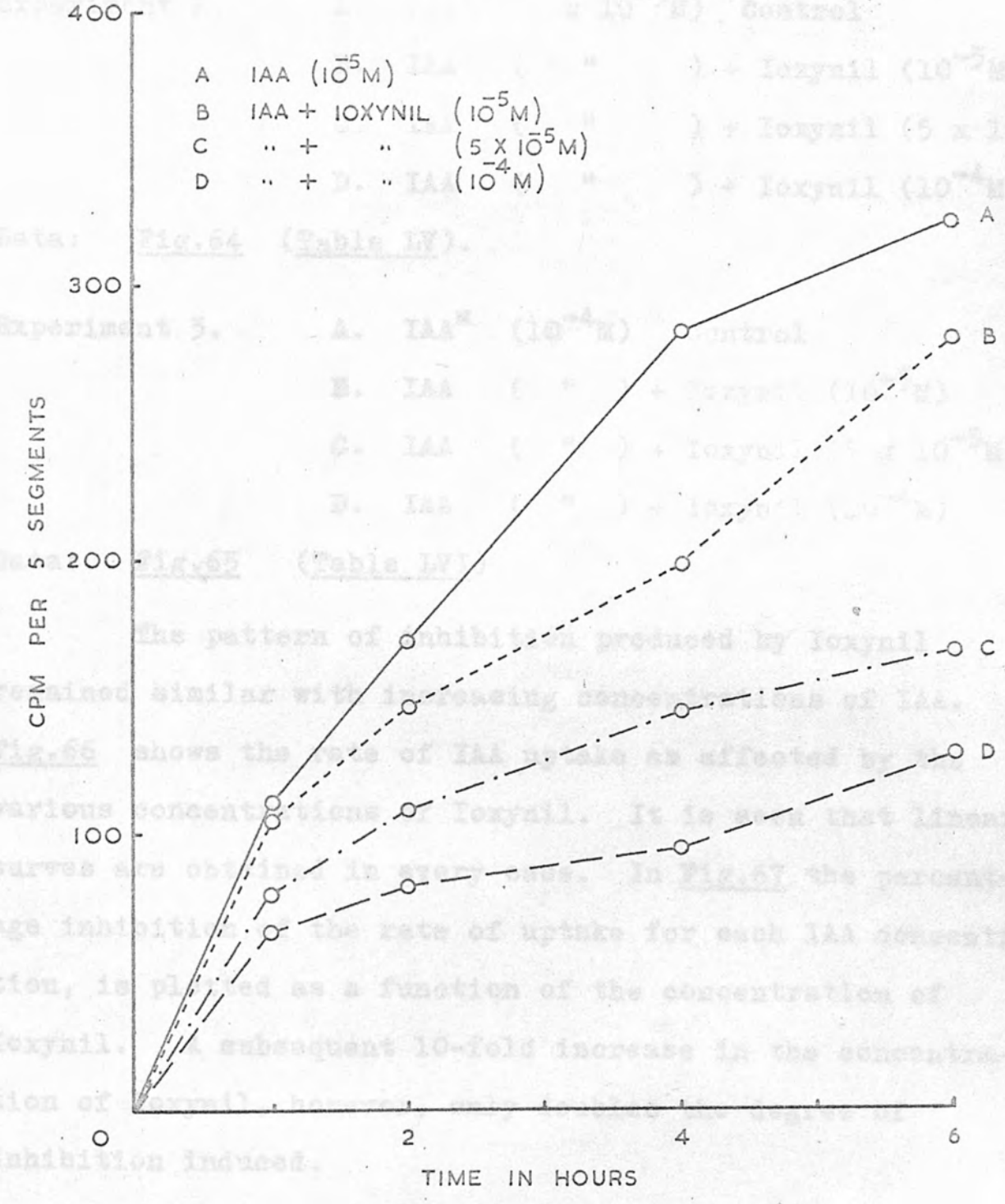
Wain (1964) recently reported the effectiveness of certain halogen-substituted benzoic acids as herbicides. These compounds are closely related to TIBA in molecular structure and were, therefore, examined for their effects on IAA uptake. The experiments are described below:

Experiment 1.
A. IAA* (10^{-5}M) Control
B. IAA (") + Ioxynil (10^{-5}M)
C. IAA (") + Ioxynil (5×10^{-5}M)
D. IAA (") + Ioxynil (10^{-4}M)

Data: Fig.63 (Table LIV)

All the concentrations of Ioxynil tested inhibited the uptake of IAA. At the low IAA concentration (10^{-5}M) the degree of inhibition exhibited by each concentration of this compound is considerably greater than that shown by TIBA in comparable experiments. There was no indication of any synergistic interaction during uptake. Though the initial phase of uptake was affected to some degree, the greater

FIG. 63


A IAA (10^{-5}M)
B IAA + IOXYNIL (10^{-5}M)
C " + " (5×10^{-5}M)
D " + " (10^{-4}M)
400
300
200
100
0
2
4
6
A
B
C
D
CPM PER 5 SEGMENTS
TIME IN HOURS

part of the inhibitory action of Ioxynil appeared to be exerted on the "metabolic" phase of IAA uptake.

Experiment 2. A. IAA* (5×10^{-5}M) Control

B. IAA (") + Ioxynil (10^{-5}M)

C. IAA (") + Ioxynil (5×10^{-5}M)

D. IAA (") + Ioxynil (10^{-4}M)

Data: Fig.64 (Table LV).

Experiment 3. A. IAA* (10^{-4}M) Control

B. IAA (") + Ioxynil (10^{-5}M)

C. IAA (") + Ioxynil (5×10^{-5}M)

D. IAA (") + Ioxynil (10^{-4}M)

Data: Fig.65 (Table LVI)

The pattern of inhibition produced by Ioxynil remained similar with increasing concentrations of IAA. Fig.66 shows the rate of IAA uptake as affected by the various concentrations of Ioxynil. It is seen that linear curves are obtained in every case. In Fig.67 the percentage inhibition of the rate of uptake for each IAA concentration, is plotted as a function of the concentration of Ioxynil. A subsequent 10-fold increase in the concentration of Ioxynil, however, only doubles the degree of inhibition induced.

FIG. 64

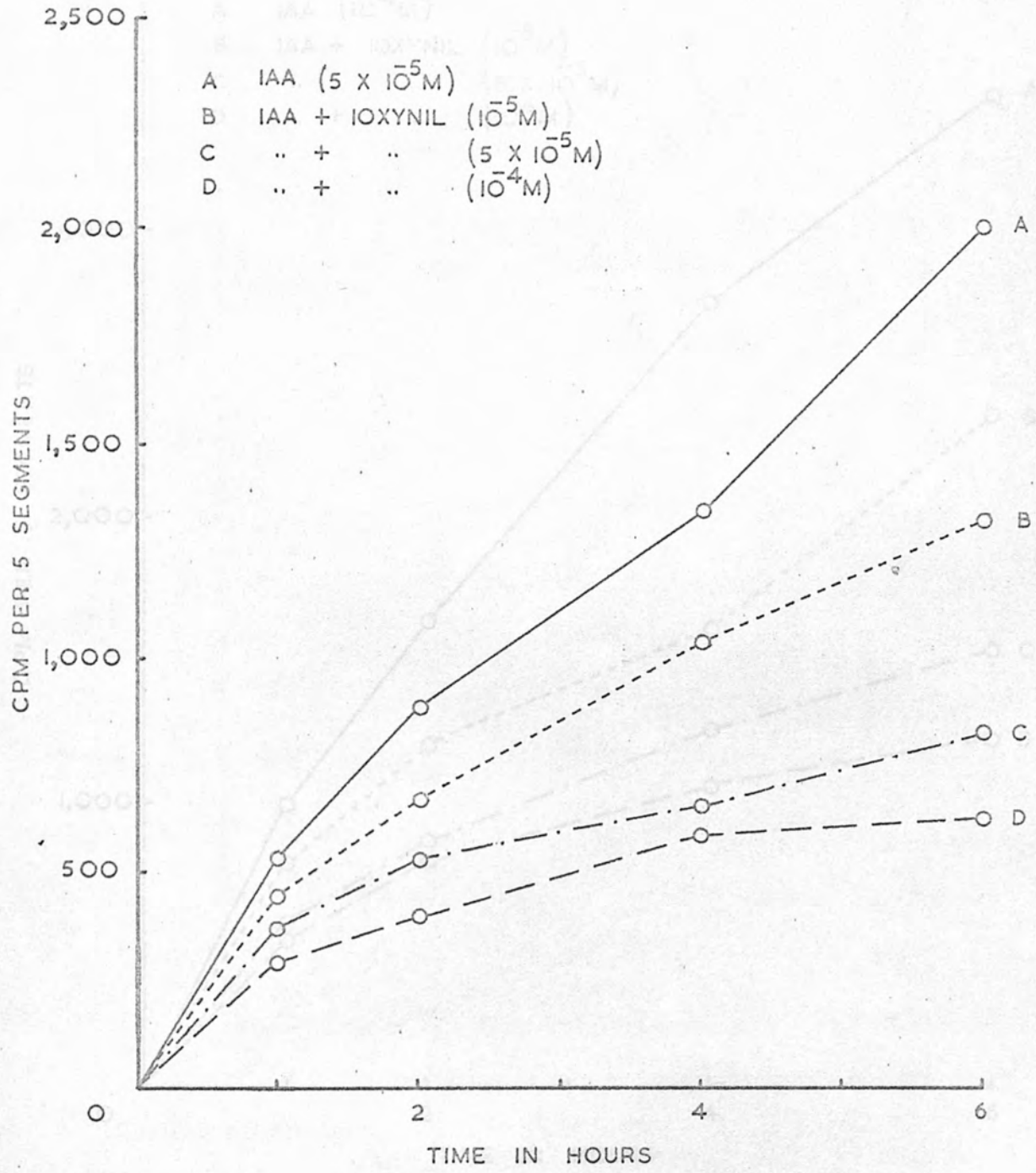

2,500
2,000
1,500
1,000
500
0
CPM PER 5 SEGMENTS
A IAA (5 X 10⁻⁵M)
B IAA + IOXYNIL (10⁻⁵M)
C " + " (5 X 10⁻⁵M)
D " + " (10⁻⁴M)
A
B
C
D
2
4
6
TIME IN HOURS

FIG. 65

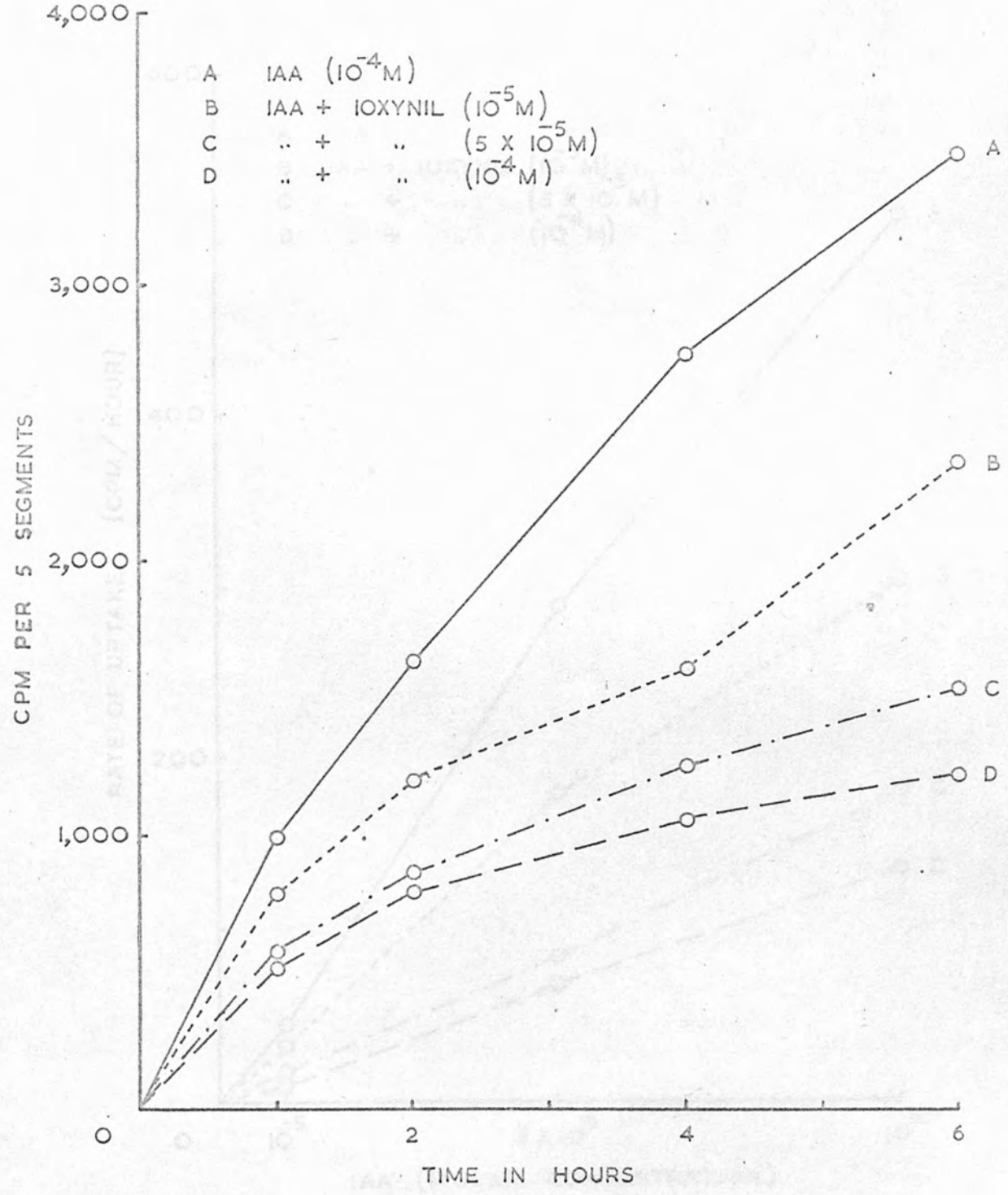

4,000
3,000
2,000
1,000
0
CPM PER 5 SEGMENTS
A IAA (10^{-4}M)
B IAA + IOXYNIL (10^{-5}M)
C .. + .. (5×10^{-5}M)
D .. + .. (10^{-4}M)
A
B
C
D
2
4
6
TIME IN HOURS

FIG. 66

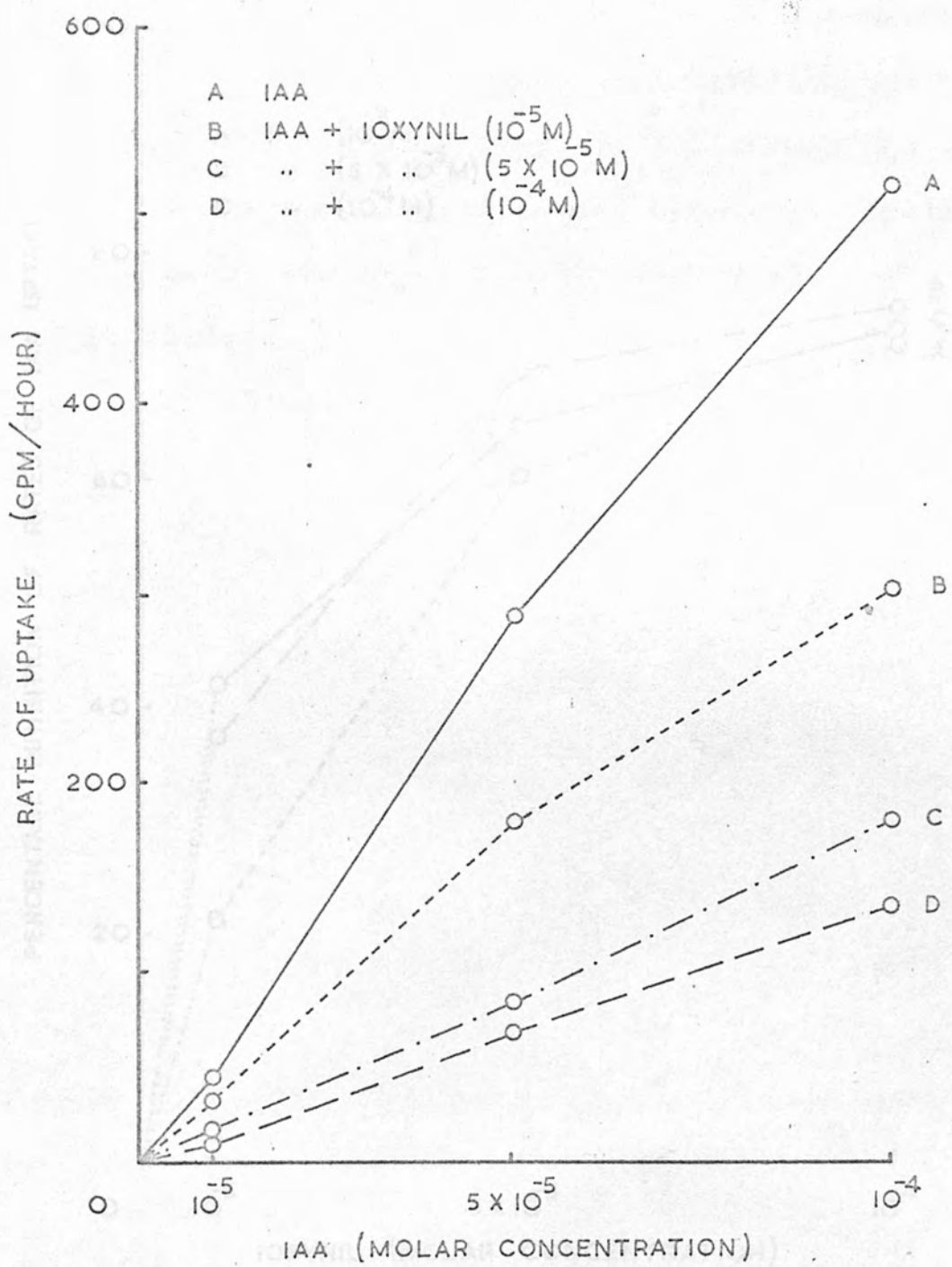
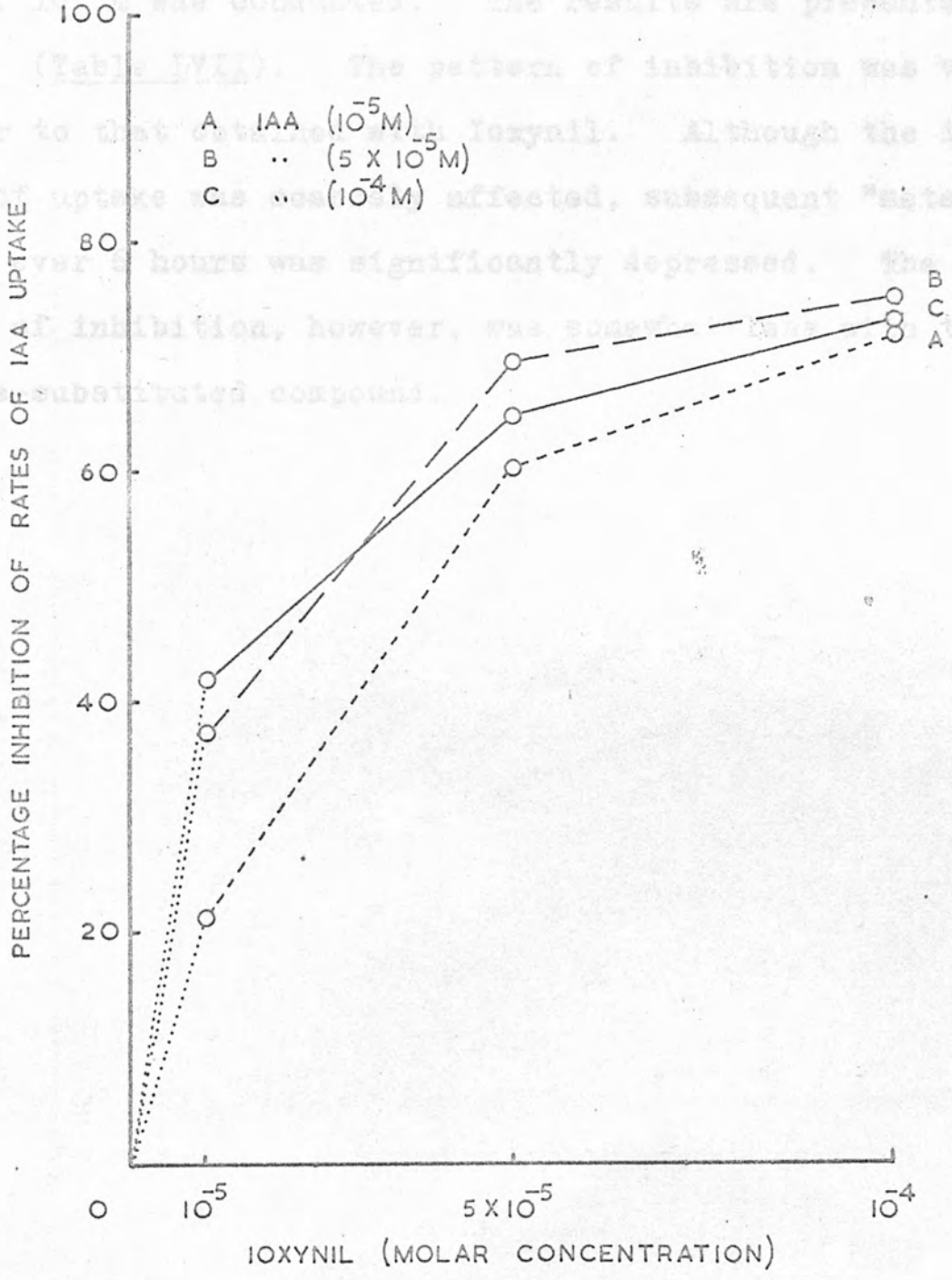
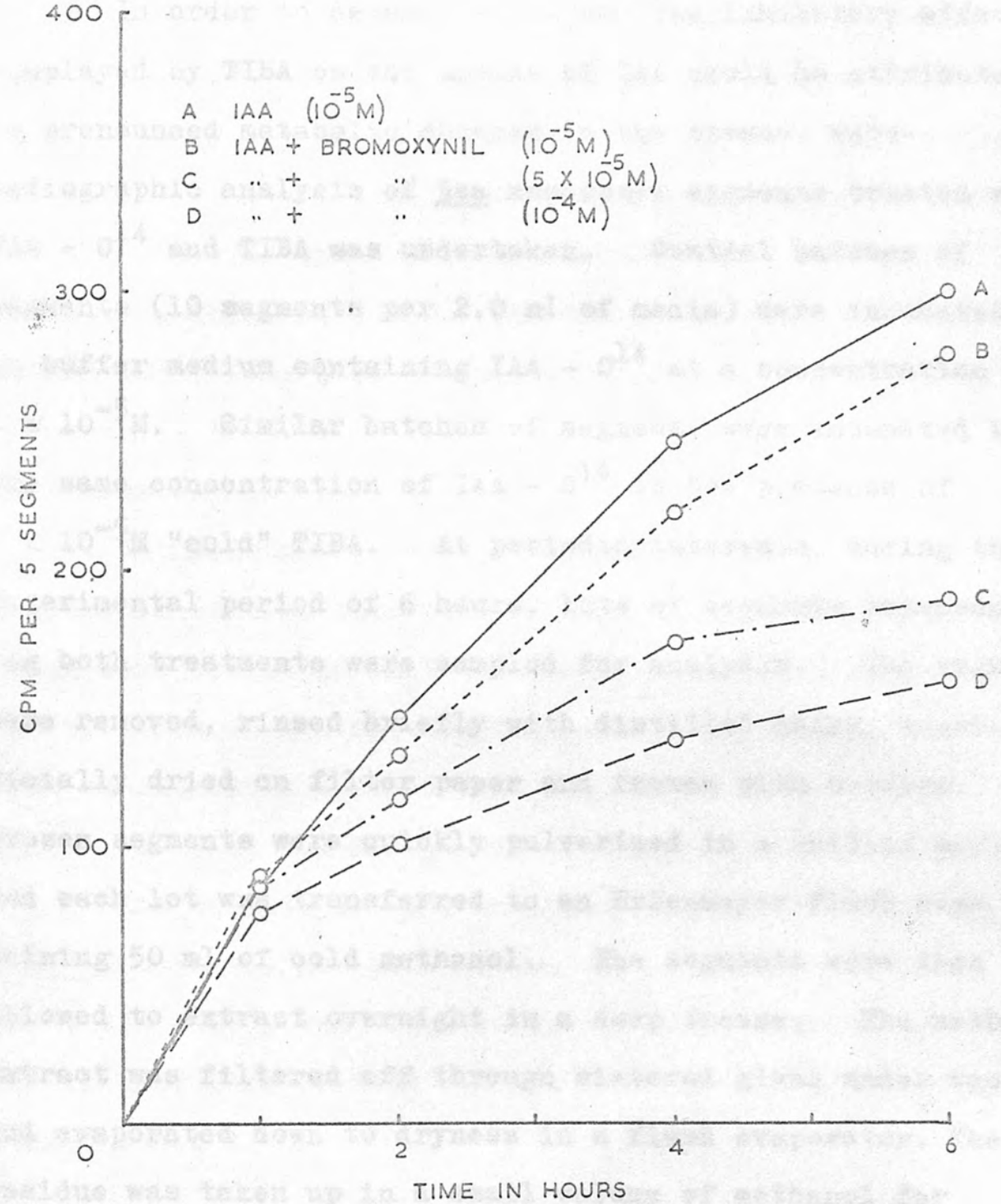

600
400
200
0
A IAA
B IAA + IOXYNIL (10^{-5}M)
C .. + .. (5×10^{-5}M)
D .. + .. (10^{-4}M)
A
B
C
D
RATE OF UPTAKE (CPM/HOUR)
10^{-5}
5×10^{-5}
10^{-4}
IAA (MOLAR CONCENTRATION)

FIG. 67


A IAA (10^{-5}M)
B " (5×10^{-5}M)
C " (10^{-4}M)
PERCENTAGE INHIBITION OF RATES OF IAA UPTAKE
100
80
60
40
20
0
10^{-5}
5×10^{-5}
10^{-4}
IOXYNIL (MOLAR CONCENTRATION)
B
C
A

BROMOXYNIL which is a bromine-substituted sister compound to Ioxynil was also examined for its effects on IAA uptake. A single experiment employing IAA* at a concentration of 10^{-5}M was conducted. The results are presented in Fig.68 (Table LVII). The pattern of inhibition was very similar to that obtained with Ioxynil. Although the initial phase of uptake was scarcely affected, subsequent "metabolic" uptake over 6 hours was significantly depressed. The degree of inhibition, however, was somewhat less with the bromine-substituted compound.

FIG. 68


400
300
200
100
0
CPM PER 5 SEGMENTS
A IAA (10^{-5}M)
B IAA + BROMOXYNIL (10^{-5}M)
C " + " (5×10^{-5}M)
D " + " (10^{-4}M)
A
B
C
D
2
4
6
TIME IN HOURS

XV

RADIOAUTOGRAPHY

In order to determine whether the inhibitory effects displayed by TIBA on the uptake of IAA could be attributed to pronounced metabolic changes in the tissue, autoradiographic analysis of Zea mesocotyl segments treated with IAA - C^{14} and TIBA was undertaken. Control batches of segments (10 segments per 2.0 ml of media) were incubated in buffer medium containing IAA - C^{14} at a concentration of 5×10^{-5}M. Similar batches of segments were incubated in the same concentration of IAA - C^{14} in the presence of 5×10^{-5}M "cold" TIBA. At periodic intervals, during the experimental period of 6 hours, lots of segments representing both treatments were sampled for analysis. The segments were removed, rinsed briefly with distilled water, superficially dried on filter paper and frozen with cardice. The frozen segments were quickly pulverized in a chilled mortar and each lot was transferred to an Erlenmeyer flask containing 50 ml of cold methanol. The segments were then allowed to extract overnight in a deep freeze. The methanol extract was filtered off through sintered glass under vacuum and evaporated down to dryness in a flash evaporator. The residue was taken up in a small volume of methanol for chromatography. The procedure for extraction and

chromatography was adapted from the methods described by Andreae, et al (1956: 1961).

The extracts of the IAA*-control and TIBA - treated tissue segments were spotted on sheets of Whatman No.1 chromatography paper along with marker spots of synthetic IAA - C^{14}. Descending chromatography was employed. The solvent selected was iso-PrOH : NH_4OH : H_2O 80 : 10 : 10 (Stowe and Thimann, 1954). The chromatograms were equilibrated for 1 hour over the solvent and then run overnight in the dark at ca.20°C. The solvent front was allowed to travel approximately 30 cms.

Autoradiograms were prepared by leaving the chromatograms in contact with Kodak X-ray film for one week. The films were developed in Kodak ID 19 developer and examined for evidence of radioactive regions. The chromatograms were subsequently sprayed with a modified Salkowski reagent (0.5M $FeCl_3$ in 10% $HClO_4$) and the reactive spots developed.

The results of this analysis are presented in Table 20. Only one radioactive spot, corresponding in Rf to IAA - C^{14}, was detected in all the chromatograms. No differences were evident between the IAA - C^{14} control and TIBA-treated tissues.

TABLE 20

Extract	Time of Sampling	Rf	Colour	Intensity Guide
A_1	1 hour	0.40	Rose Madder	Faint
B_1	"	0.42	"	"
A_2	2 hours	0.39	"	"
B_2	"	0.40	"	"
A_4	4 hours	0.42	"	Medium
B_4	"	0.47	"	"
A_6	6 hours	0.49	"	"
B_6	"	0.48	"	"
IAA-C^{14}	_____	0.45 - 0.53	"	Strong

A : Zea mesocotyl segments incubated in IAA - C^{14} (10^{-4}M):

B : " " " " " IAA - C^{14} (10^{-4}M) + TIBA (10^{-4}M):

The solvent front was allowed to travel 31.0 cms.

Colour Reagent: Modified Salkowski.

XVI

DECARBOXYLATION OF IAA - C^{14}

In order to examine whether the apparent inhibitory effects of TIBA on IAA uptake were the result of a stimulation of IAA-oxidase activity, measurements were made of the degree of decarboxylation exhibited by Zea mesocotyl segments treated with IAA* and IAA* plus TIBA. Two control batches of 10 segments each were incubated for a period of 5 hours in IAA - C^{14} solution at a concentration of 10^{-4}M, in 1" x 1" glass vials. Two similar batches were floated on a medium containing IAA* at 10^{-4}M and an equimolar concentration of TIBA.

To measure respiratory $C^{14}O_2$, Daly, et al (1962) and Daly (1963) employed a pyrex cylinder sealed with a rubber stopper. From the stopper was suspended a 1"-diameter stainless steel planchet. The planchet contained 0.1 ml of 10% KOH and a disc of lens paper which served to provide a uniform layer of KOH. To measure $C^{14}O_2$ output, the planchets were rapidly dried at 80°C and counted directly. Romberger and Norton (1961) incubated their plant material in flasks closed with rubber stoppers equipped with hooks and loops of Whatman No.1 paper saturated with 10% NaOH. Exposed papers were eluted with water and aliquots assayed for C^{14} by a barium carbonate precipitation technique.

The carbonate was plated on discs of Whatman No.50 paper which were pasted on to aluminium planchets. Sample thickness was uniform (below 0.4 mg/cm^2) and self-absorption was negligible.

For the present work a similar technique was adapted. The vials containing the plant material and bathing solutions were stoppered with air-tight polythene caps. Small hooks of stainless steel wire were fused to the inner surface of the stoppers. Circles of Whatman No.1 paper were punched out to the diameter of the aluminium planchets used, saturated with 20% KOH and suspended within the experimental vials by means of the hooks. During the experimental period the vials were gently agitated at frequent intervals. At the end of 5 hours the discs of filter paper were removed, fitted into weighed aluminium planchets smeared with a trace of gum solution and quickly dried in an oven at 90°C. The planchets were then reweighed and counted in the Betamat for at least 400 seconds. The mean counts obtained were corrected for background and are expressed as counts per minute in Table 21. C^{14} - activity was extremely low, indicating very little oxidative degradation of IAA under the experimental conditions. TIBA had no marked effect on the degree of decarboxylation.

TABLE 21

6

IAA	TIBA	Total Uptake (in CPM)	$C^{14}O_2$ evolved (in CPM)
10^{-4}M	____	2,700	41.0
			48.0
10^{-4}M	10^{-4}M	1,100	38.0
			36.0

10 segments per 2.0 ml of the experimental solutions.

XVII

MATERIAL: Phaseolus - hypocotyl

METHOD: Fluorescence assay

GROWTH-SUBSTANCE: IAA

Uptake of IAA by segments from the hypocotyl of Phaseolus radiatus L. (Mung bean) was also examined by fluorescence assay and measurements of radioactivity.

In a preliminary experiment, lots of 20 hypocotyl segments were incubated for three hours in buffer and three concentrations of IAA ranging from 10^{-5}M to 5 x 10^{-5}M. At periodic intervals the solutions were sampled and examined for fluorescence. The fluorimetric values obtained for the experiment are given in Table 22. Although some slight fall in the fluorescence intensity of the IAA solutions was noted, more noticeably with the higher concentrations, the decrease was unexpectedly low. Samples from the buffer alone in which segments were floated indicated a startling rise in the intensity of emission. The activation and emission spectra were carefully examined and appeared to be identical with those for pure IAA.

A further experiment was therefore conducted to examine this phenomenon more closely. The number of segments was increased from 20 to 50 per 10 ml of solution.

TABLE 22

MEDIA	FLUORESCENCE INTENSITY					
	In.(a)	In.(b)	30 m	1 hr	2 hr	3 hr
BUFFER	0.10	0.10	0.36	0.50	0.78	0.90
IAA (10^{-5}M)	2.55	2.70	2.81	2.80	2.65	2.65
" (2.5×10^{-5}M)	6.95	6.68	7.00	6.80	6.68	6.00
" (5×10^{-5}M)	14.55	13.95	13.80	13.20	12.60	10.95

Uptake of IAA by mung bean hypocotyl segments in terms of decreasing fluorescence intensity. 20 segments per 10 ml solution.

The tissue was equilibrated in distilled water or buffer for 30 minutes prior to transference into the experimental solutions. The sampling was extended over a period of 6 hours.

Experiment 2.

A. Buffer

B. Distilled water

C. IAA (10^{-5}M) in Buffer

D. IAA (10^{-5}M) in Distilled water.

The fluorimetric readings are graphically represented in Fig.69 (Table LVIII). Whereas IAA is quite readily taken up from distilled water, no such uptake is measurable from buffer. In the latter case, although some slight uptake is indicated during the first hour, no further losses in fluorescence intensity were recorded thereafter.

When the segments are incubated in buffer alone, there is a steep rise in the intensity of fluoresence recorded at the same excitation and emission maxima as IAA. It was determined by careful checks that this was not due to scattering of any kind. Samples from distilled water media in which segments were floated indicated a small but significant rise in fluorescence intensity during the first hour, which remained constant for three hours and then gradually declined. The results seem to indicate that the

FIG. 69: UPTAKE OF IAA

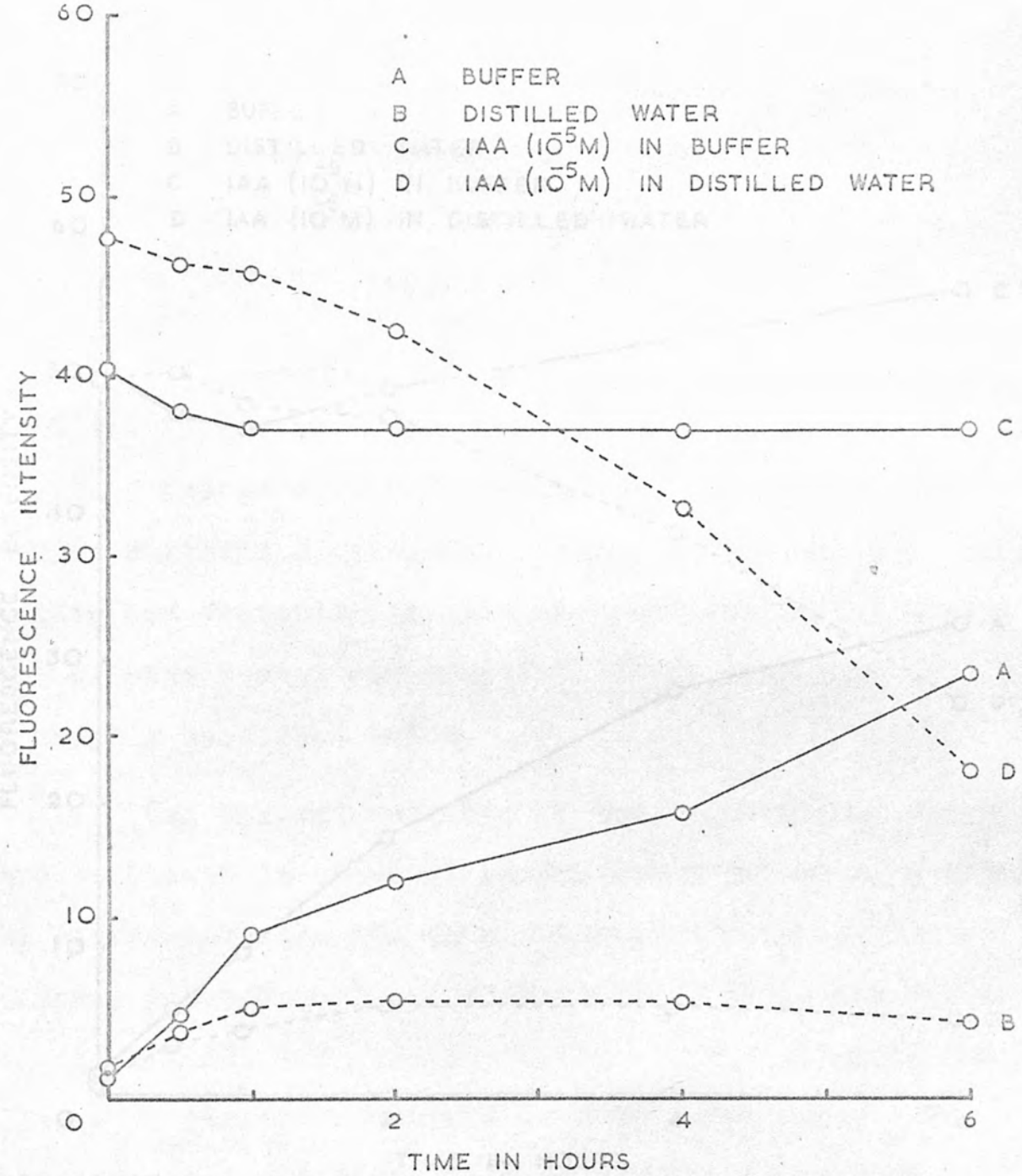
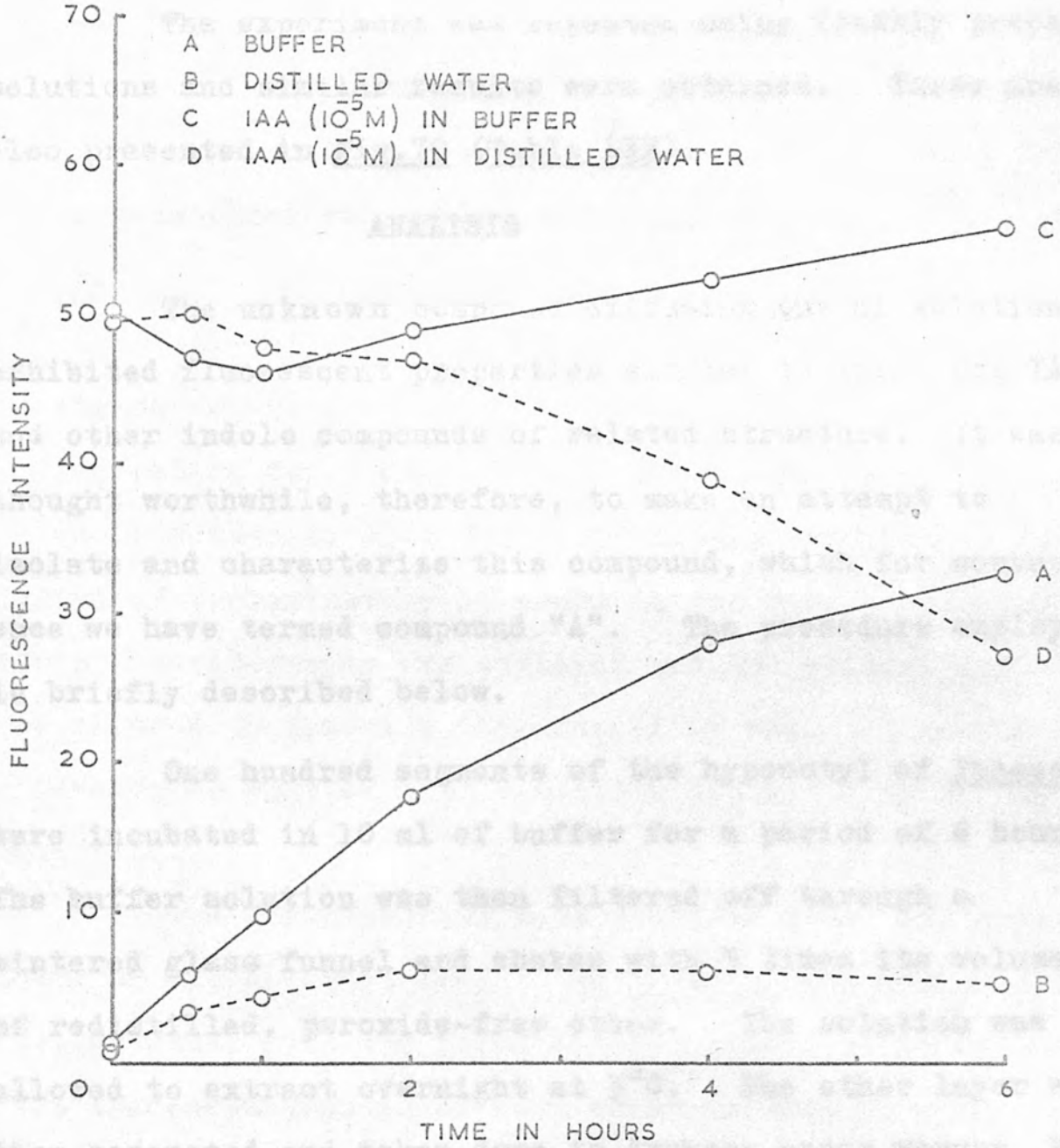


FIG. 70: UPTAKE OF IAA



fluorescence values obtained for uptake of IAA from buffer or distilled water are the additive results of exchange of IAA from the external solution for the unknown fluorescing compound.

The experiment was repeated using freshly prepared solutions and similar results were obtained. These are also presented in Fig.70 (Table LIX).

ANALYSIS

The unknown compound diffusing out of solution exhibited fluorescent properties similar to those for IAA and other indole compounds of related structure. It was thought worthwhile, therefore, to make an attempt to isolate and characterize this compound, which for convenience we have termed compound "A". The procedure employed is briefly described below.

One hundred segments of the hypocotyl of Phaseolus were incubated in 10 ml of buffer for a period of 6 hours. The buffer solution was then filtered off through a sintered glass funnel and shaken with 3 times its volume of redistilled, peroxide-free ether. The solution was allowed to extract overnight at 3°C. The ether layer was then separated and taken down to dryness under vacuum. The residue was taken up in a small volume of redistilled

methanol and stored in a deep freeze for analysis. This will be designated as Extract A.

The hypocotyl segments, after the buffer medium had been filtered off, were transferred to a small, conical flask, covered with 25 ml of freshly distilled ether and allowed to extract overnight. The ether layer was then filtered, washed with a small volume of water, taken down to dryness under vacuum and the residue taken up in a small volume of methanol for analysis (Extract B).

Aliquots of both extracts, A and B, were spotted on strips of Whatman No.1 filter paper and run, after equilibration for 1 hour, in a solvent comprising of iso-butanol: methanol: H_2O: NH_3 (S.D. 0.880) - 80:5:15:1 for a period of approximately 14 hours in the dark. Ascending strip chromatography was employed and the solvent front was allowed to travel a distance of 25 cms. A marker chromatogram spotted with synthetic IAA was also run in the same tank.

The chromatograms were removed, dried and examined under an ultra-violet lamp. In Table 23 are included the data obtained from the three chromatograms. It is evident that the chromatograms of both extracts show no evidence of any region corresponding to the marker IAA. The chromatogram of extract B was sprayed with the Salkowski

TABLE 23

EXTRACT	Spot No.	Rf	Colour under Ultra Violet	Intensity Guide
A	1	0.05	Bright Blue	
	2	0.28	Purplish - Blue	Faint
	3	0.54	Purplish - Blue	Very Faint
	4	0.65	Yellow	Very Faint
	5	0.90	Blue	Strong
B	1	0.14	Bright Purple-Blue	
	2	0.19	Yellow	Faint
	3	0.83	Orange - Yellow	Strong
	4	0.91	Blue	Strong
IAA Standard		0.40	Blue	

Chromatograms examined under Ultra - Violet light.

A - Extract of Buffer medium.

B - Extract of Mung bean hypocotyls.

reagent (Gordon and Weber, 1951) but no positive reaction was obtained.

The chromatogram of extract A was divided into 25 segments of 1 cm each. Each segment was transferred to a vial and eluted with 2.0 ml of distilled water for 1 hour. The eluates were decanted into centrifuge tubes and spun at 3,000 rpm to remove particles of paper. Each eluate was then carefully examined in the spectrophotofluorimeter for the presence of fluorescing compounds. Thorough scanning, however, failed to detect any significant traces of fluorescence. The unknown compound which interferes with the assay of IAA uptake was clearly absent and must be presumed to have been lost or destroyed during the analytical procedure.

EFFECT OF pH ON THE FLUORESCENCE OF THE DIFFUSATE FROM MUNG BEAN HYPOCOTYL.

It is known that a change in the pH of the medium can affect the fluorescence of a compound by causing a shift in the wavelength of the fluorescence maximum, by changing the intensity of fluorescence, or by a combination of these two effects. Udenfriend, Weissbach and Clark(1955) and Gally and Edelman (1962) have demonstrated characteristic shifts in the emission wavelengths of the 5-hydroxy indoles,

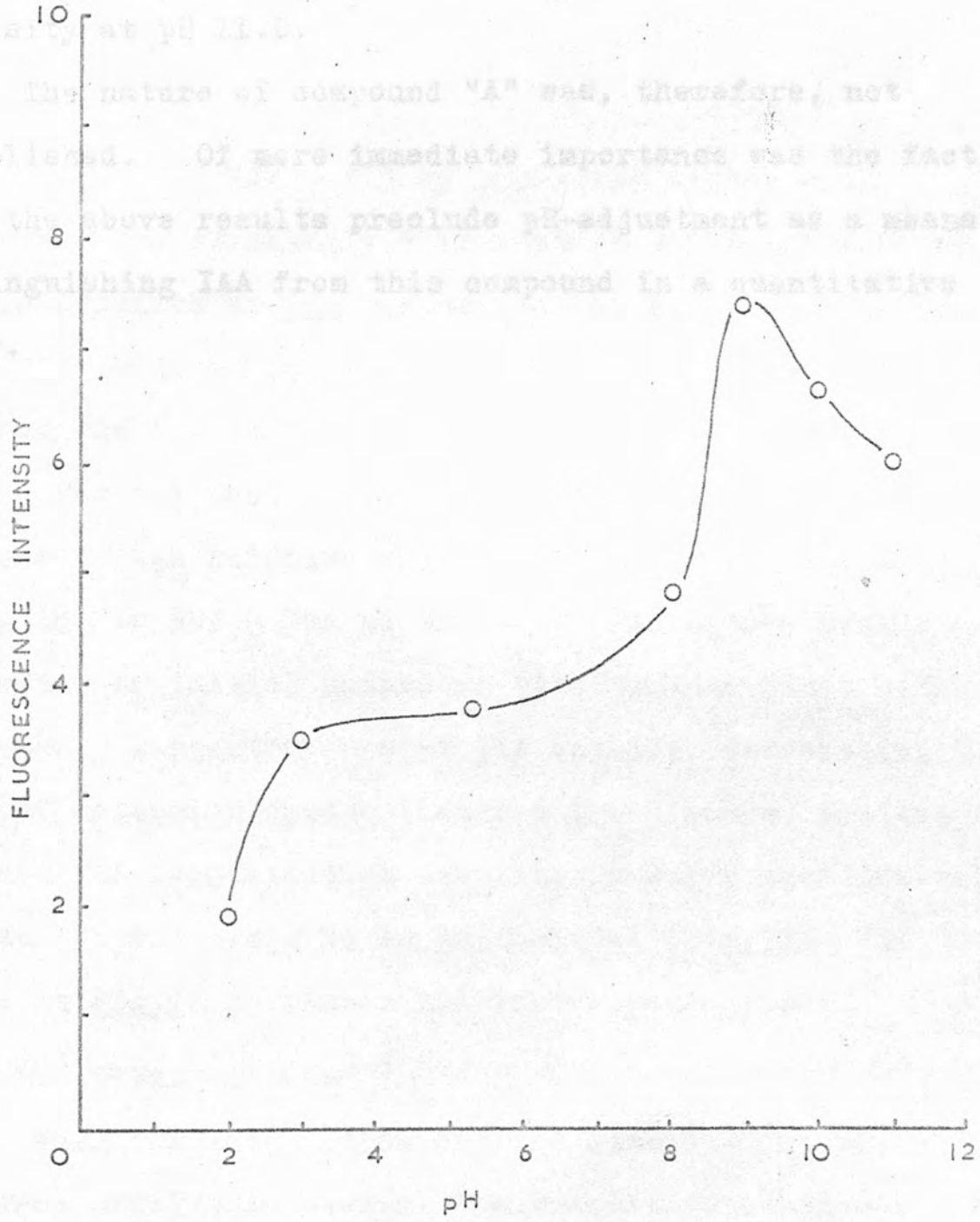
tryptophan and related compounds in highly acid or alkaline solutions. Burnett and Audus (1964) discuss in some detail the effect of changing pH on the fluorescence intensity of a number of indole compounds. It has been shown that the differences in behaviour between these compounds is so marked that this effect can be used to discriminate between them.

In the present work, a brief examination of the effects of pH on the fluoresecnec intensity of compound "A" was carried out. One hundred hypocotyl segments were incubated in 10 ml of buffer for 6 hours. 2.0 ml samples of the buffer medium were then adjusted to varying pH values ranging from 2.0 to 11.0 with $N/_1$ HCl and $N/_{10}$ NaOH. In Fig.71 the fluorescence intensity of compound "A" (at excitation and emission maxima as for IAA) has been plotted against the pH of the samples. The values are derived from the results of two experiments.

The curve shown in Fig.71 has a sharp peak at pH 9.0. With decreasing alkalinity there is a steep fall which levels off to a plateau between pH 7 and pH 3 then falls sharply again. Of the 22 indole compounds examined by Burnett and Audus (1964) only 2, tryptophan and N-methyl-tryptophan, showed similar curves. Tryptophan, however, is activated

FIG. 71: EFFECT OF pH ON FLUORESCENCE OF COMPOUND 'A'

EMISSION MAX: 284 MμL
FLUORESCENCE MAX: 365 MμL



at 295 mμ and fluoresces at 340 mμ, while N-methyl-tryptophan is activated at 280 mμ and fluoresces at 355 mμ. Both compounds demonstrate a sharp peak of maximum fluorescence intensity at pH 11.0.

The nature of compound "A" was, therefore, not established. Of more immediate importance was the fact that the above results preclude pH-adjustment as a means of distinguishing IAA from this compound in a quantitative assay.

XVIII

MATERIAL: Phaseolus - hypocotyl

METHOD: Radioactivity assay

GROWTH-SUBSTANCES: IAA - C^{14}

IAA* + TIBA

Uptake of IAA - C^{14} by hypocotyl segments of Phaseolus was strikingly different from the patterns of uptake afforded by Zea mesocotyls and Avena coleoptiles. The results of an experiment employing a range of IAA concentrations (10^{-5}M to 10^{-4}M) are presented in Fig.72 (Table LX). For all concentrations of IAA applied there is an extremely high relative absorption over the first hour amounting to 50% - 75% of the total IAA uptake over 6 hours. The ratio of initial uptake to total uptake rises with increasing concentrations of IAA applied. Conversely, the rate of metabolic uptake (between 1 - 6 hours) plotted against IAA concentration describes a curve that gradually slopes off and seems to be exponential (Fig.73). The dotted lines in Fig.72 represent the linear regressions.

IAA* + TIBA:

When the interaction of TIBA with IAA* uptake was examined using this tissue, the results obtained were still more unexpected. Three concentrations of TIBA

FIG. 72: UPTAKE OF IAA-C^{14}

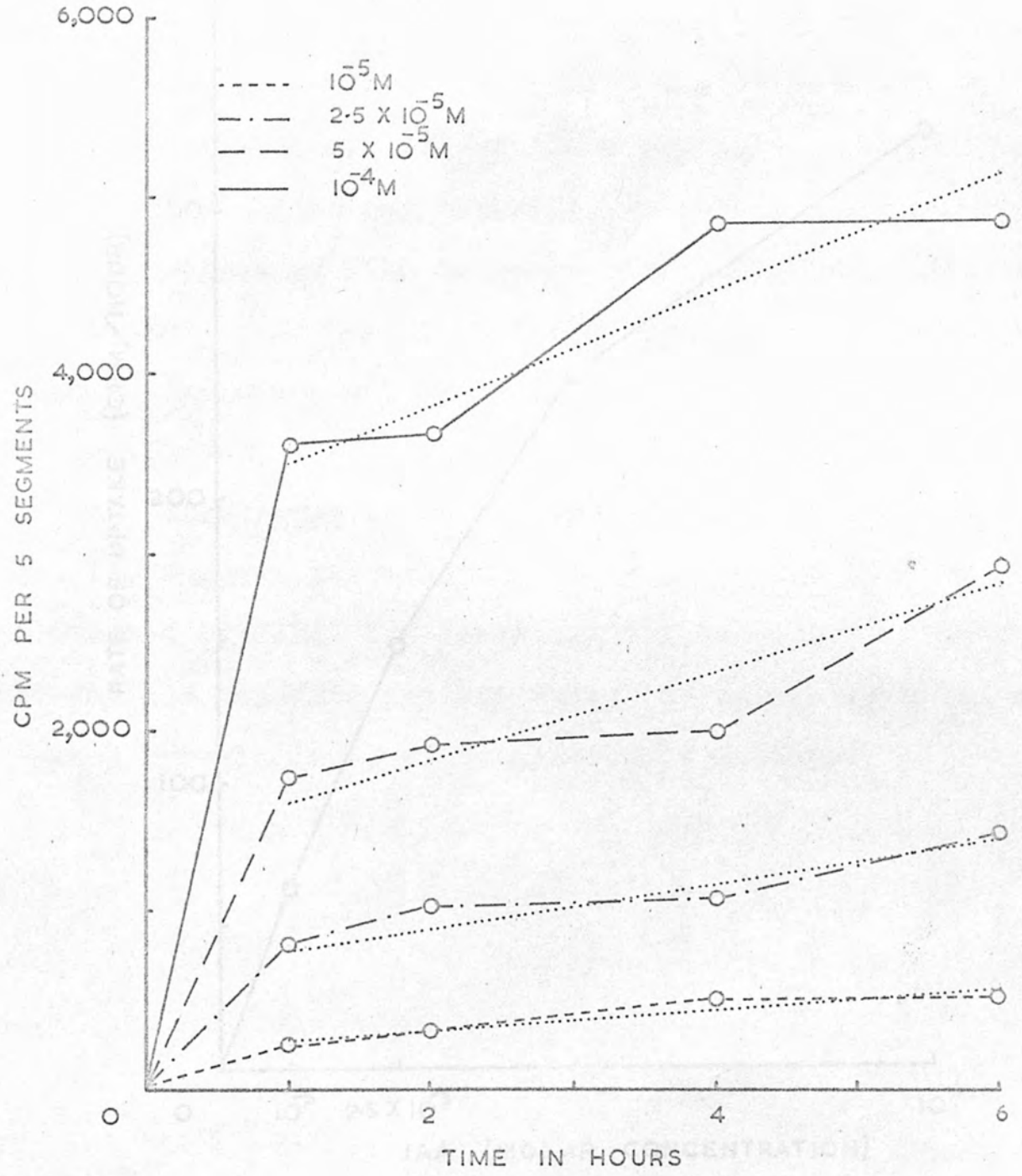
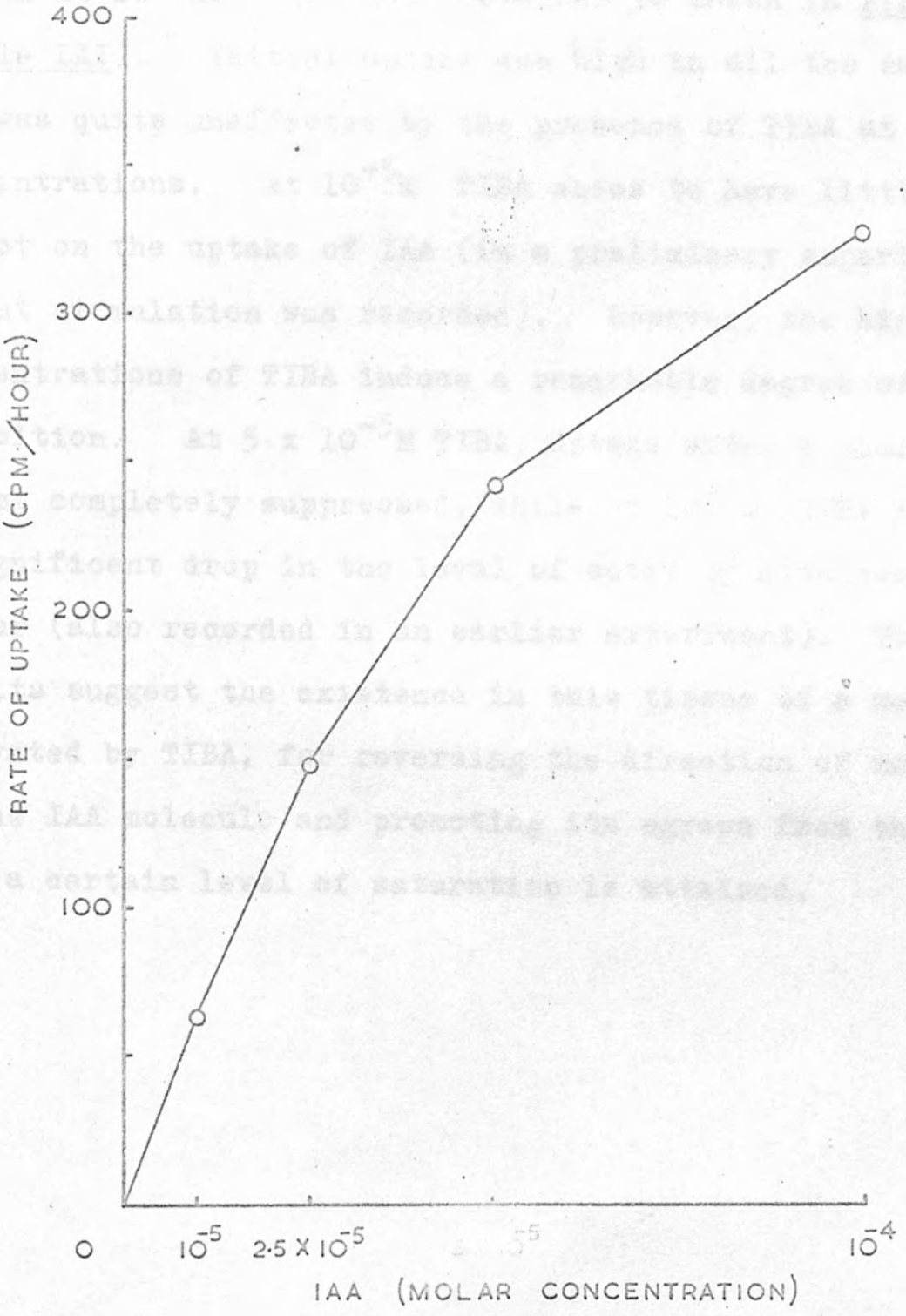
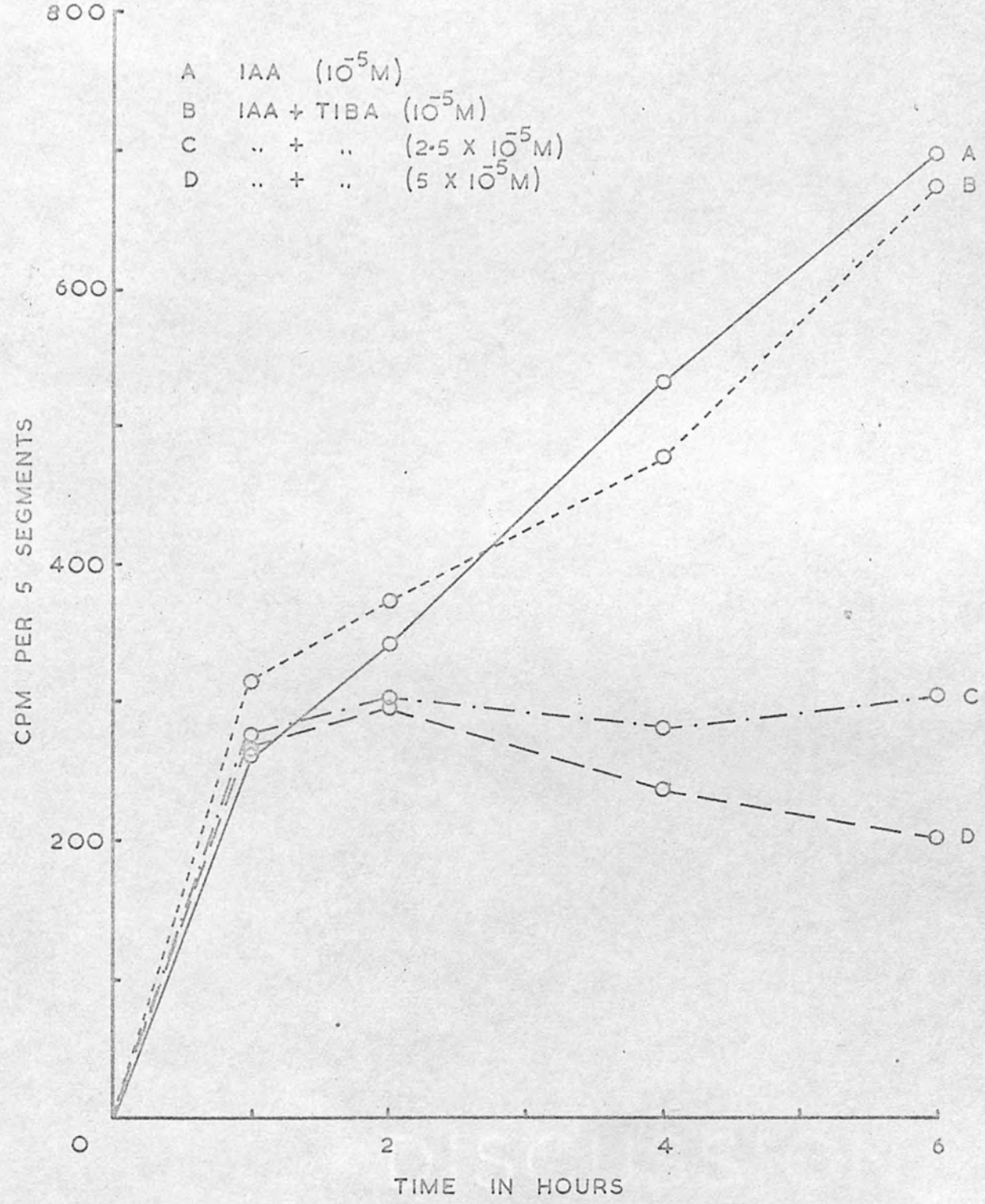


FIG. 73


400
300
200
100
0
RATE OF UPTAKE (CPM/HOUR)
10^{-5}
2.5×10^{-5}
10^{-4}
IAA (MOLAR CONCENTRATION)

(10^{-5}M to 10^{-4}M) were tested for their effects on the uptake of IAA at 10^{-5}M. The data obtained ~~is~~ are shown in Fig.74 (Table LXI). Initial uptake was high in all the samples and was quite unaffected by the presence of TIBA at all concentrations. At 10^{-5}M TIBA seems to have little effect on the uptake of IAA (in a preliminary experiment some slight stimulation was recorded). However, the higher concentrations of TIBA induce a remarkable degree of inhibition. At 5 x 10^{-5}M TIBA, uptake after 1 hour is almost completely suppressed, while at 10^{-4}M, TIBA induces a significant drop in the level of activity attained after 1 hour (also recorded in an earlier experiment). The results suggest the existence in this tissue of a mechanism, activated by TIBA, for reversing the direction of movement of the IAA molecule and promoting its egress from the tissue when a certain level of saturation is attained.

FIG. 74


800
600
400
200
0
2
4
6
CPM PER 5 SEGMENTS
TIME IN HOURS
A IAA (10^{-5}M)
B IAA + TIBA (10^{-5}M)
C .. + .. (2.5×10^{-5}M)
D .. + .. (5×10^{-5}M)
A
B
C
D

DISCUSSION

DISCUSSION

The net influx of a solute may be controlled by the balance between the rate at which it enters the cell and the rate at which it is metabolized. The active transport system can be considered to be not only the mechanism responsible for accumulation of the solute within the cell, but also the force which moves the compound across a permeability barrier. Thus, in some cases, once the solute has entered the cell, the rate of metabolism or chemical binding to a cellular constituent may be such that movement into the cell is never against an activity gradient.

The coupling between metabolism and the mechanisms responsible for active transport is suggested to be phosphorylative. This link may be direct or indirect. Sulfhydryl groups might also be widely involved (Tanada,1956).

The accumulation of some compounds appears to be the net result of inward and outward fluxes which may differ in their respective properties. Intracellular concentrations of the solute may also influence the rate of absorption from the external medium.

Uptake of IAA:

Early workersin the field (Albaum, et al, 1937:

Sutter, 1940; Reinhold, 1954) estimated the uptake of auxin by plant tissue using methods for the chemical determination of indoleacetic acid disappearance from the external medium. The sensitivity of these methods limited the range of external auxin concentrations to 10^{-4} M and greater. This is well above the "physiological" range of auxin concentrations which promotes the growth of Avena coleoptile segments, pea stems and other tissues.

Johnson and Bonner (1956), using radioactive 2,4-D, were able to work at concentrations as low as 0.1 mg/litre. Their results indicated that the uptake of 2,4-D by Avena coleoptile sections seems to involve three separable components in the process. These are respectively: (1) a diffusion into an accessible fraction of the tissue, (2) an exchangeable binding, and (3) a continuing uptake. Poole and Thimann (1964) also showed that Avena coleoptile segments rapidly take up IAA - C^{14} from solution during the first half hour, and then accumulate the compound, against a concentration gradient, at a nearly linear rate for 4 hours.

In the present work, however, neither fluorescence assay of the external solution, nor measurements of radioactivity absorbed, indicated any suggestion of two distinct phases during the uptake of IAA by Avena coleoptile segments.

Pretreatment of the tissue with a metabolic poison (sodium cyanide) demonstrated that the uptake of IAA, after one hour of incubation, may be almost completely suppressed. On the other hand, uptake during the first hour is only partially sensitive to cyanide. The results suggest that this initial phase of absorption consists of a physical process, probably simple diffusion, which is unaffected by the cyanide treatment, and a second process, possibly active, which is cyanide-sensitive. Whether the latter process can be equated with the exchangeable binding system, described by Johnson and Bonner, for 2,4-D uptake by Avena is open to argument. These workers repressed both the initial and continuing uptake of 2,4-D with similar concentrations of cyanide.

Studies on the uptake of IAA by mesocotyl segments of Zea revealed that uptake during the first 30 minutes was clearly measurable and relatively rapid. The IAA absorbed during the first hour accounted for about 25% of the total accumulated at the end of 6 hours.

For a given concentration of IAA in the external medium, the rate of uptake describes a tendency to decline with time. This may be largely attributed to the fall in the external concentration as uptake progresses. The

above conclusion is supported by the results of the experiment in which mesocotyl segments, pretreated with cold IAA for a period of 4 hours, were transferred to a solution of IAA - C^{14} of the same initial concentration. Uptake of IAA - C^{14} continued at the same rate as that exhibited by control segments incubated directly in a medium of IAA - C^{14}.

For about 2 - 4 hours after the plant tissue was immersed in the auxin solution, net uptake of IAA was virtually proportional to the external concentration. Subsequently, however, the relative decline in the rate of uptake was enhanced by increasing IAA concentrations. The mean rate of uptake, between 1 hour of incubation and the termination of the experiment, when plotted as a function of IAA concentration, demonstrates a curvilinear relationship. This curve describes a tendency to flatten that becomes more pronounced as the concentration of IAA exceeds the physiological range. These observations, therefore, point towards the existence of a system responsible for the active uptake of IAA, which is gradually saturated and slowed down by increasing concentrations of the auxin accumulated within the tissue.

That the continuing uptake of IAA is an active process, at least partially dependent on some form of respiratory energy, is further suggested by the significant depression in the rate of absorption induced by the phosphorylative uncoupling agent, 2, 4-dinitro-phenol. Johnson and Bonner (1956) demonstrated a similar effect of DNP on 2,4-D uptake by Avena coleoptiles. Both auxin-induced growth and the uptake of salts were inhibited by DNP. The inhibitory effect of DNP on growth, however, was far more pronounced than its effect on uptake.

Robertson (1951) suggested that ion-accumulation by plants is closely linked to the cyanide-sensitive fraction of respiration. On the other hand, Reinhold (1954) showed that the metabolic uptake of IAA by carrot tissue discs was considerably more sensitive to cyanide than was the cyanide-sensitive respiration.

Swets and Wedding (1964) found that the curve representing uptake of 2,4-D by Chlorella, under the influence of increasing concentrations of DNP, showed a valley and a peak. They distinguished (a) a process increased by uncoupling concentrations of DNP, and (b) a process inhibited by DNP at concentrations even lower than those causing severe uncoupling. They suggested that the process stimulated

by DNP is glycolysis resulting in the accumulation of pyruvate and other closely related compounds. The inhibitory effect they ascribe to an effect of DNP on a reaction associated with the conversion of pyruvate to acetyl CoA.

The continuing uptake of 2,4-D by Avena coleoptile segments was depressed by 33% after pretreatment for 1 hour, with 3×10^{-5} M DNP (Johnson and Bonner, 1956). In the present work, pretreatment with 10^{-4} M DNP inhibited total uptake at the end of 6 hours, by ca. 25% . It is evident, therefore, that even high concentrations of DNP do not completely suppress metabolic uptake. It is suggested, therefore, that a smaller fraction of the metabolic energy required for active uptake is derived from oxidative phosphorylation and is consequently DNP - sensitive. A larger fraction, which is unaffected by DNP but sensitive to cyanide, may possibly be contributed by glycolysis.

It may be mentioned in passing that Niedergang-Kamien and Leopold (1957) have shown that concentrations of DNP (10^{-4}M) which increased the respiration of sunflower stem tissue, inhibited auxin transport by 75%. With higher concentrations, which inhibited respiration, auxin transport was completely suppressed.

Further evidence to suggest that steady state uptake is an active process is the apparent accumulation of IAA against a concentration gradient in the tissue. Poole and Thimann (1964) found that when Avena coleoptile segments were floated on a solution of IAA, between 1 and 2 hours, the internal concentration of IAA exceeded the external concentration. Subsequent uptake showed no signs of slowing down within 4 hours. They conclude that true accumulation occurs.

In the table below, the weight, [illegible] of an Avena coleoptile segment is compared with similar data for a Zea mesocotyl segment.

Tissue	Wt. (gms)
Avena	0.0119
Zea	0.0384

The volume of water in 100 ten - mm. Avena coleoptile segments is approximately 1.1 cc. Therefore, when they are incubated in 10 ml of IAA solution, the concentration of IAA within the tissue may be assumed to equal that of the external solution when 11% of the total IAA in the external solution is absorbed. In actual fact, 11% is the widest margin, the accessible space within the tissue being considerably less

in all probability. Nevertheless, this state was attained within 1 to 2 hours, agreeing closely with the results of Poole and Thimann.

By contrast the volume of water in 50 ten - mm. mesocotyl segments is approximately 1.6 cc. In this tissue the internal concentration of IAA equals the external concentration in about 2-3 hours.

An anatomical examination showed that the cells of the coleoptile are smaller and more numerous. The intercellular spaces are also smaller. It is suggested, therefore, that IAA reaches a concentration equilibrium in the accessible free space of the tissue, faster in the case of the Avena coleoptile as against the Zea mesocotyl, because of the smaller proportion of this accessible volume of tissue in the former material.

A second possible explanation for this phenomenon may be based on the transport factor. Jacobs (1961) showed a clear positive relation between the number of cells in a bean hypocotyl section and the amount of auxin that the section transported. Graphing the data of Went and White (1939) for auxin transport in the Avena coleoptile, with

cell-length data derived from Avery and Burkholder (1936), Jacobs showed that a similar relationship existed in this tissue - more cells correlate with more transport. It is tentatively put forward that the more rapid uptake of IAA by Avena coleoptile segments might be in part associated with the greater number of cells per unit volume of tissue. In physiological terms this would imply that auxin uptake is a function of the activity of cell membranes.

Radioautographic and chromatographic analysis failed to demonstrate the presence of any products of IAA - C^{14} metabolism in mesocotyl tissue that had been incubated in a solution of IAA - C^{14} for a period of 6 hours. When segments, pretreated with radioactive IAA for 4 hours, were transferred to an equimolar solution of non-radioactive IAA, approximately 40% of the accumulated C^{14} activity was lost to the external solution. These observations support the contention that a large proportion of the IAA absorbed is not bound behind a permeability barrier or otherwise transformed within the tissue. An accumulation of IAA against a concentration gradient is, therefore, clearly suggested.

The pattern of IAA uptake described by hypocotyl segments of Phaseolus differed strikingly from those offered by Zea or Avena segments. The rate of uptake during the

first hour is relatively extremely high. The amount absorbed in this period accounts for 50 % to 75 % of the net uptake over 6 hours. In terms of counts per minute, 50 hypocotyl segments of Phaseolus take up as much IAA in the first hour as 50 Zea mesocotyl segments absorb in 6 hours. It may be mentioned that the volume of the hypocotyl segment is roughly intermediate between the Avena coleoptile and the Zea mesocotyl. After the first hour, the rate of IAA uptake by the hypocotyl segments drops strikingly.

Thus a steep gradient of distinction between the initial phase of uptake and the subsequent metabolic uptake of IAA is exhibited by the three tissues examined - no discernible distinction in Avena, a small but clear difference in Zea, and a greatly exaggerated distinction in Phaseolus.

UPTAKE OF NAA:

The uptake of NAA by Avena coleoptile tissue follows a pattern similar to that described for IAA uptake by this tissue. The rate of uptake during the initial phase, however, is clearly greater than the subsequent metabolic rate of uptake. The latter, in the case of NAA, is a linear function of external concentration over the range

examined. Net uptake, for equimolar concentrations, is, also, significantly higher for NAA than for IAA.

Leopold and Lam (1961) have shown that NAA is transported in a polar manner through sunflower stem sections, and exhibits a velocity of movement of about 7 mm/hr as against 8 mm/hr for IAA. This would suggest that similar mechanisms exist for the uptake and transport of the two compounds. The differences in the rate of uptake, observed in the present work, may well be due to factors concerned with the penetration of the molecules into the tissue.

UPTAKE OF TIBA:

The uptake of TIBA - I^{131} by Zea mesocotyl segments also follows a pattern very similar to that obtained for IAA uptake by this tissue. Over the range of concentrations examined, the rate of uptake was directly proportional to the external TIBA concentration. However, the importance of ascertaining the magnitude of absorption of this compound, in terms of absolute amounts was unfortunately overlooked in the experimental work.

Blackman and Sargent (1959) examined the uptake of TIBA by Chlorella. In higher plant tissue, although it has been

shown to be an effective inhibitor of transport, there is little evidence to show that TIBA itself is transported or accumulated. In the present investigation, therefore, there is little reason to believe that its absorption is not a passive process, either in the nature of simple diffusion or some form of facilitated diffusion.

2, 4-D - Auxin Interactions:

Johnson and Bonner (1956) showed that both the exchangeable binding and the continuing uptake of 2,4-D are inhibited in the presence of a high concentration of IAA. Uptake of 2,4-D is less influenced by this compound than is the 2,4 -D - induced growth of the Avena coleoptile.

Reinhold (1954) found that the metabolic uptake of IAA by pea epicotyl segments and carrot discs was inhibited in the presence of a high concentration of 2, 4-D (4×10^{-3}M). There was no indication that increasing IAA concentrations decreased the severity of the inhibition, and she suggested that the inhibition was non-competitive.

Wedding and Blackman (1961) found that the uptake of 2,4-D by Chlorella was depressed by IAA in a competitive manner.

2,4-D has been shown to inhibit the polar transport of IAA (Leopold and Lam, 1961). 2,4-D, itself, exhibits polarity of movement in bean petiole segments (McCready, 1963a, 1963b).

In the present work it was found that 2,4-D,at concentrations ranging from 10^{-5}M to 5 x 10^{-5}M, had no effect whatsoever on the uptake of either IAA or NAA by Avena coleoptile segments. Similarly, the uptake of IAA by maize mesocotyl segments remained unaffected. It is suggested that the inhibitory effects recorded by Reinhold are due to side effects induced by the supra-optimal concentration of 2,4-D used.

The apparent lack of any effect of 2,4-D on IAA uptake is rather surprising in view of the fact that this compound is readily absorbed and accumulated by Avena coleoptile segments (Johnson and Bonner, 1956). That there must be some common link in the mechanisms responsible for the uptake of both compounds is indicated by the inhibitory effects of IAA on 2,4-D uptake. However, it is also clear that such uni-directional inhibitory effects are not uncommon, as is exemplified by the uptake interactions of IAA and TIBA.

NMSP - Auxin Interactions:

Aberg (1950, 1951, 1952) suggested that NMSP acted as a competitive auxin antagonist in restoring the growth of roots inhibited by NAA, IAA and 2,4-D.

Audus and Das (1955) found that the curves relating root growth response to $\log_{10}$ concentration, for IAA and NMSP, were of very similar shape. In multifactorial experiments involving stimulatory concentrations of IAA with several stimulatory concentrations of NMSP, consistent mutual antagonisms were demonstrated. The results were considered to suggest that both auxins and antiauxins, in stimulating root growth, are exerting identical physiological actions in the same growth system.

Audus and Brownbridge (1957) suggested that the mutual antagonisms shown between NMSP and the auxins IAA and 2,4-D in the geotropic responses of seedling pea roots, are best explained in terms of an interference with access to the growth centres. Competitive action at the growth centres themselves seems not to be involved.

Libbert (1962) concluded from his experiments on the growth of buds on decapitated pea plants, that NMSP is a competitive antagonist of an endogenous inhibitor present in pea stems and furnished by roots and green leaves.

Ng and Audus (1964) found that NMSP antagonizes competitively the promotive action of both IAA and GA on the extension of first internode segments of Avena seedlings.

It was found in the present work that NMSP did induce some apparent inhibition of IAA uptake by Avena coleoptile segments. The degree of inhibition increased with increasing concentrations of NMSP. Increasing the concentration of IAA did not appear to relieve this inhibitory effect. The results rule out the possibility of any form of competitive antagonism between the two compounds during uptake. However, the data obtained from replicate experiments varied considerably and reproducibility was low. That it would be unwise to draw any definite conclusions from the above results, is further supported by the lack of any observed effects of NMSP on the uptake of NAA by Avena tissue or on the uptake of IAA by Zea mesocotyl segments.

If one accepts the suggestion of Audus and Brownbridge that the antiauxin effect of NMSP is not exerted at the growth centre, then the possibility arises that its antagonism is effected through an interference with the translocation of IAA from the tissue surface to the locus of the growth reaction. There has been no published work on the effect of NMSP on IAA transport, and this suggestion remains hypothetical. It is clear, however, that the

penetration and accumulation of IAA and NAA are little affected.

NPA - Auxin Interactions:

Mentzen and Netien (1950) found that NPA induced negative geotropic responses in a variety of species. Netien and Conillot (1951) showed that NPA is entirely without detectable antagonistic effect on IAA response in the standard Avena test, although it exhibits a variety of other antiauxin effects.

Grigsby, et al (1954) found that treatment of pea seedlings with NPA resulted in 70% reduction of straight growth and a complete loss of geotropic sensitivity. Ching, et al (1956) also recorded a disproportionate inhibition of growth and curvature in NPA-treated roots and shoots of corn and pea, but no disproportionality in NPA-treated Avena coleoptiles.

Uptake studies revealed that NPA, tested over a range of concentrations, stimulated the uptake of IAA by Zea mesocotyl segments. It was noted that this effect is more pronounced at low interacting concentrations of the two compounds and is reduced or completely overcome by increasing concentrations of one or both compounds.

Audus (unpublished) also recorded stimulatory effects of NPA on extension growth, both in the absence and presence of added IAA. There is a distinct possibility, therefore, that NPA exerts its stimulatory effect on IAA uptake, through facilitating the transport of IAA within the tissue. The effects of this compound on transport phenomena are certainly worthy of more attention.

TIBA - Auxin Interaction:

Galston (1947) suggested that the effects of TIBA may be mediated by the auxin system, and showed that it could considerably reduce or inhibit the effect of IAA in the Avena curvature test. The effect of TIBA in promoting abscission or modifying leaf shape (Waard and Florschutz, 1948) could be counteracted by the simultaneous application of IAA. In the pea test, Thimann and Bonner (1948 a) found a positive effect of TIBA alone (5×10^{-5}M and 10^{-4}M) which disappeared, however, if the slit sections were washed for 2 hours in water before the experiment, suggesting the TIBA effect to be mediated by the residual auxin in the stem. In the pea test, the Avena coleoptile straight growth test and in the Avena curvature test, they found the effects of low IAA concentrations to be enhanced by

low concentrations of TIBA, inhibitory effects appearing at higher concentrations. Muir and Hansch (1951) reported slight positive effects of TIBA alone (5×10^{-7}M to 5×10^{-5}M) on the elongation of Avena coleoptile sections.

Åberg (1950, 1953) obtained similar responses with TIBA in the growth of roots. Low concentrations of this compound (10^{-8} - 10^{-6}M) displayed synergistic effects with the native auxin. At higher concentrations (10^{-6} - 10^{-5}M) the antagonistic effects became considerable. At still higher concentrations (10^{-5} - 10^{-4}M) toxic effects set in. They found, however, that no synergism was obtained with TIBA and low concentrations of added 2,4-D. Åberg suggested that an explanation of this synergistic effect of TIBA might be sought in its action on the enzymic systems regulating IAA metabolism in plant cells. Thimann and Bonner (1948) and Veldstra and Booij (1949) postulated that cases of synergistic action between growth substances could be explained on the basis of competition by the synergist with auxins for inactive positions in the plasm, leaving more auxin molecules free to occupy active sites. Audus (1954) suggested that the synergistic action of

low concentrations of TIBA and IAA in the inhibition of root growth, reported by Åberg, may be a purely additive effect. Gorter (1962) proposed that apparent synergistic effects between auxin and other compounds in a growth response might be through the effect of the synergist on the penetration of the auxin.

In the results of the present investigation, there is much evidence to indicate that low concentrations of TIBA stimulate the uptake of IAA by maize mesocotyl segments. Whether this effect is exerted on the penetration of the compound or through a metabolic reaction is not clear.

The stimulation of IAA uptake by both NPA and TIBA may be visualised as being exerted through a mechanism whereby the penetration is facilitated of the auxin molecules through the outer permeability barrier of the cell, presumably the plasmolemma. At higher concentrations, inhibitory effects may then be exerted through interference in a metabolic reaction, perhaps associated with the accumulation of IAA within the cell. Such inhibitory effects would eventually mask the lesser action on penetration.

A number of workers have demonstrated that TIBA

effectively blocks the transport of IAA in a variety of tissues (Zwar and Rijven, 1956; Hay, 1956; Niedergang-Kamien and Skoog, 1956; Niedergang-Kamien and Leopold,1957; Leopold and Lam, 1961; Hertel and Leopold, 1963). Niedergang-Kamien and Skoog suggest that many of the physiological effects shown by TIBA may be ascribed to the inhibition of polar auxin transport. Niedergang-Kamien and Leopold report that this inhibition of transport polarity is specific and more pronounced than the effects on respiration. Kessler and Moscieki (1958), however, have presented indirect evidence to indicate that TIBA promotes the translocation of calcium and iron in plants.

If one accepts the proposition that "metabolic" uptake is a function of a transport system based on a carrier mechanism of some sort, it is difficult to equate the inhibitory effects of TIBA on the transport of IAA, reported in the literature, with the stimulatory effects exerted by low TIBA concentrations on IAA uptake observed in this investigation. It is possible, however, that TIBA may effect its inhibitory action at some intermediate state in the process, - perhaps before the postulated static auxin pool is filled and movement of auxin through the tissue is activated.

It is equally possible that the inhibitions induced by TIBA on IAA uptake are the consequence of interference in the sequence of metabolic events responsible for the accumulation of IAA in the tissue. Åberg, in a series of publications (1950, 1951, 1952a, 1952b, 1953) reported the effects of a number of auxin antagonists on the root growth of flax seedlings, and suggested that their effects could be explained on the basis of competition for receptive sites within the cell. A number of other workers have explained the mechanism of auxin action and interaction on the basis of competition for reactive sites based on the structural configurations and chemical affinity of the growth substances.

Thimann and Bonner (1948a, 1948b, 1949a), examining the effects of various inhibitors and organic acids on the growth of the Avena coleoptile, concluded that the growth process is controlled by an enzyme or coenzyme containing a sulfhydryl group. Inhibitions could be effected through the affinity of the compounds tested by them for this SH-group. Leopold and Guernsey (1953b) proposed a theory to explain the mechanism of auxin action which involved the formation of a thio-ester between auxins and coenzyme A. A number of other workers have also explored the possibility

that an SH-enzyme system may be involved in auxin action (Veldstra and Havinga, 1943; Thimann, 1951; Mayer and Evanari, 1951; Millard and Bonner, 1954; Leopold, 1955; Leopold and Price, 1957). Thimann and Bonner (1949b) concluded that coumarin and protoanemonin inhibit growth through reacting with a sulf-hydryl enzyme, and that this enzyme is normally a limiting factor in growth. The inhibition is prevented by the addition of the SH-enzyme protector 2,4-dimercaptopropanol (British Anti-Lewisite—BAL) at concentrations below 3×10^{-4}M.

It is evident from the results presented in the previous chapter, that BAL and cysteine have no effect on the TIBA-induced inhibition of IAA uptake. This would seem to indicate that a sulfhydryl enzyme system is not involved in the mechanism responsible for TIBA action on uptake. The results also indicate that the TIBA-induced inhibitions are much more pronounced than those evidenced by 2,4-dinitrophenol.

Autoradiographic and chromatographic analysis failed to reveal any IAA - C^{14} metabolites or detoxication products formed in control and TIBA-treated tissue. Measurements of respiratory $C^{14}O_2$ indicated that oxidative decarboxylation of IAA - C^{14} was extremely low in both control and

treated segments, and no significant differences were recorded between them.

The effect of TIBA on the uptake of IAA by hypocotyl segments of Phaseolus is even more difficult to interpret in terms of an uptake interaction between the two compounds. The blockage of uptake at high concentrations of TIBA is sudden and complete. The induced loss of IAA from the tissue is likely to be simple diffusion along a concentration gradient, as it seems fairly certain that uptake at the end of one hour has moved the IAA into the tissue against an activity gradient.

Effect of Ioxynil and Bromoxynil on IAA Uptake:

Wain (1963, 1964) has reported the strong herbicidal activity of the di-halogenated - hydroxynenzonitriles. These were found to be effective for a wide variety of weeds. The di-iodo compound proved more effective than the di-bromo compound, the least active being the chlorine-substituted derivative.

The iodo- and bromo- compounds were also found to inhibit the uptake of IAA very markedly in the present study. Ioxynil exhibited a greater inhibitory action than Bromoxynil.

UPTAKE MECHANISMS:

From the preceding discussion it may be clear that much evidence has accumulated to support the view that auxin uptake is an active process, possibly consisting of more than one component in the system. The system possesses many of the generally accepted characteristics of an active transport mechanism - (a) a high temperature coefficient (demonstrated by earlier workers), (b) accumulation against a concentration gradient, (c) the rate of absorption is different for substances of similar molecular size and lipoid solubility, although they may be structurally related, and (d) inhibition of absorption is possible with a variety of enzyme inhibitors and poisons. A fifth criterion for active uptake is competition for entry between similar substances, in a manner similar to competitive inhibition of enzyme activity. These interactions may be analysed in terms of Michaelis-Menton enzyme kinetics. In the present work, however, no such competition for entry was observed.

The suggestion that the auxin uptake system, under the conditions in which such studies have been conducted so far, may comprise more than one active component, is thought to be supported by the following argument. In the hypocotyl of Phaseolus, the enormous influx of auxin during

the first hour, moving against an activity gradient, cannot be accounted for by physical factors. That the subsequent slow uptake of IAA can be completely suppressed is demonstrated by the experiments with TIBA. A transport factor may be involved in the latter process. However, it is likely that a large proportion of the initial influx of IAA into this tissue is governed by extremely high metabolic activity in the superficial layers of cells adjoining the cut surface. The IAA may, therefore, be absorbed from the external medium, as a consequence of the wound effect, by an uptake system supplementary to the normal carrier mechanism existant in the tissue. The activity of such a system may, of course, vary extensively from tissue to tissue.

AUXIN KINETICS:

In all the work carried out with naturally-occuring and synthetic growth substances, no reaction between the auxins and any chemically-defined entity in the cell has been shown to be a part of the sequence of events culminating in a growth reaction. In an attempt to elucidate this point, Bonner and co-workers,in a series of publications (McRae and Bonner, 1952, 1953; Foster, McRae and Bonner, 1952,1955; Foster and Niemann, 1953; McRae, Foster and Bonner, 1953)

applied a scheme of "auxin kinetics", identical in formulation with the enzyme kinetics of Michaelis and Menten (1913) and Lineweaver and Burk (1934) to the analysis of the cell elongation that occurs when segments of oat coleoptiles elongate under the influence of auxins. Induced differences in the growth response by different growth substances have been treated by kinetic analysis for competitive interaction of substrates at a common enzymic site.

McRae and Bonner (1953) developed a hypothesis in which auxin - antiauxin actions in growth have been related to the molecular configuration of these applied substances. The two-point attachment concept developed by Muir and Hansch (1951, 1953, 1955) has been accepted as the basis on which the hypothesis has been built. McRae and Bonner calculated the equilibrium constants (K_S and K_I) and the free energy of formation (by the method described by Dixon,1953) for the establishment of some auxin-antiauxin complexes with Avena coleoptile sections. These parameters permit the determination of the affinities of each interaction point of the auxin molecule for its respective group on the receptor entity. The concept was extended to explain

growth inhibition at supraoptimal auxin concentrations (Foster, McRae and Bonner, 1952), and to antiauxin action at high auxin concentrations (Foster, McRae and Bonner, 1955).

Housley (1961) has presented an extensive critical review of the applications and limitations of the various forms of auxin kinetics applied to the interaction of growth substances evaluated by a growth response. Several other workers (Audus, 1954; Bennet-Clark and Kefford, 1954; Burstrom and Hansen, 1956), have questioned the validity of these kinetic concepts on a variety of grounds. The objections include the assumption that endogenous growth substances regulate growth in excised sections, the application of auxin kinetics to what in the growth process must be multi-enzyme systems, factors of penetration,etc.

Housley, Bentley and Bickle (1954) and Housley (1961) have pointed out that internal concentrations of applied regulators at receptor sites are not necessarily identical with external concentrations initially applied. Studies on auxin transport have shown that the movement of IAA and other auxins is inhibited by the presence of various growth substances, metabolic inhibitors and other factors

in different ways and to different degrees. This transport factor is, therefore, capable of masking the true values of the parameters associated with enzyme-substrate affinity. Similar arguments have been applied to the effect of the permeability of the tissue to different molecules. The conjugation and detoxication mechanism that have been shown to exist in tissues have also to be considered when applying auxin kinetics to the growth process. Thus a number of factors must be taken into consideration before any assumption is made relating intracellular and external concentrations of applied growth substances.

The results of the present work support these arguments in so far as it makes clear that internal auxin concentrations cannot be simply equated with external concentration. It is also obvious that such direct kinetic treatment cannot be applied to the uptake interactions of the various auxins and antiauxins examined in this investigation.

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APPENDIX

Note 1 : Fluorescence assay - uptake is expressed as micrograms of the growth substance per 100 Avena coleoptile segments, 50 Zea mesocotyl segments or 50 Phaseolus hypocotyl segments, except where specified.

Note 2 : Radioactivity Assay - uptake is expressed in terms of counts per minute (CPM) per 5 segments.

Note 3 : Except where specified, the rate of uptake has been calculated from the values for uptake between 1 hour and the termination of the experiment.

Note 4 : Fluorescence intensity is represented in arbitrary units of the photometer scale.

Abbreviations :

M.D. = Mean Deviation.

% I/S = Percentage inhibition or stimulation of the rate of IAA uptake.

In (a) = Initial measurement of fluorescence intensity taken just prior to introducing the plant material.

In (b) = Experiment sampled and measured immediately after immersing the tissue.

TABLE I

		UPTAKE OF IAA (µg)					
		15 m	30 m	1 hr	2 hr	4 hr	6 hr
10^{-5}M IAA in Distilled Water	Expt. 1	0.48	0.03	0.18	1.76	4.43	5.79
	Expt. 2	0.21	1.13	2.04	2.16	4.42	6.10
	MEAN	0.35	0.58	1.11	1.96	4.42	5.95
	M.D.	0.14	0.55	0.93	0.20	0.00	0.16
10^{-5}M IAA in Buffer	Expt. 1	0.77	0.04	1.01	2.33	4.52	6.12
	Expt. 2	0.31	-0.28	0.06	0.80	3.27	5.45
	MEAN	0.54	-0.12	0.54	1.56	3.90	5.78
	M.D.	0.23	0.16	0.48	0.77	0.62	0.33

Uptake of IAA by Avena coleoptile segments from distilled water and buffer media respectively.

TABLE II

IAA	TIME	IAA UPTAKE (IN MICROGRAMS)			
		Expt. 1	Expt. 2	MEAN	M.D.
5×10^{-6}M	15 m	0.40	-0.03	0.19	0.21
	30 m	0.02	0.19	0.11	0.09
	1 hr	0.47	-0.07	0.20	0.27
	2 hr	0.93	0.43	0.68	0.25
	4 hr	2.36	2.06	2.21	0.15
10^{-5}M	15 m	0.92	0.00	0.46	0.46
	30 m	0.05	0.21	0.13	0.08
	1 hr	0.29	0.46	0.38	0.09
	2 hr	1.71	1.73	1.72	0.01
	4 hr	3.85	3.65	3.75	0.10
2.5×10^{-5}M	15 m	0.42	0.00	0.21	0.21
	30 m	0.02	0.00	0.01	0.01
	1 hr	1.05	1.48	1.26	0.22
	2 hr	4.66	4.76	4.71	0.05
	4 hr	7.64	9.12	8.38	0.74
5×10^{-5}M	15 m	0.00	1.38	0.69	0.69
	30 m	1.46	1.90	1.68	0.22
	1 hr	5.35	6.57	5.96	0.61
	2 hr	11.99	12.79	12.39	0.39
	4 hr	20.40	22.24	21.32	0.92

Uptake of IAA by Avena coleoptile segments.

TABLE III

Concentr. of IAA	Expt. No.	Rate of Uptake	Mean Rate of Uptake
5×10^{-6}M	1	0.64	0.68
	2	0.72	
10^{-5}M	1	1.17	1.11
	2	1.05	
2.5×10^{-5}M	1	2.10	2.30
	2	2.49	
5×10^{-5}M	1	4.90	5.03
	2	5.16	

Rate of uptake of IAA expressed as micrograms IAA per 100 Avena coleoptile segments per hour.

TABLE IV

TIME	UPTAKE OF IAA (µg) A	B	C	D	E	MEAN	M.D.
1 hour	0.80					0.80	___
2 hour	1.12	1.48				1.30	0.18
4 hour	3.94	4.40	4.55			4.29	0.24
6 hour	5.62	6.44	5.41	5.68		5.79	0.33
8 hour	8.51	9.34	8.58	8.68	7.73	8.57	0.36

Effect of sampling on the uptake of IAA by Avena coleoptile segments.
A - E = Lots of 100 segments in 10 ml of 10^{-5} M IAA solution.

TABLE V

Expt. No.	IAA	NaCN	Uptake of IAA (µg)					Rate of Uptake	% I
			15 m	30 m	1 hr	2 hr	4 hr		
1	10^{-5}M	____	____	0.00	0.47	1.23	3.62	1.07	
	"	____	____	0.00	0.58	1.70	4.12	1.19	
	"	10^{-5}M	____	0.14	0.77	1.83	4.39	1.22	0.00
	"	10^{-4}M	____	-0.21	-0.01	1.07	3.17	1.06	5.86
2	10^{-5}M	____	0.06	0.55	0.71	1.20	3.79	1.06	
	"	10^{-5}M	-0.32	-0.27	0.26	1.33	3.33	1.02	4.14
	"	10^{-4}M	0.00	-0.43	-0.43	0.65	2.49	0.96	9.40

Effect of NaCN pretreatment on the uptake of IAA by Avena coleoptile segments.

TABLE VI

Expt. No.	IAA	NaCN	UPTAKE OF IAA (µg)					RATE	% I
			15 m	30 m	1 hr	2 hr	4 hr		
3	10^{-5}M	____	0.48	0.11	1.61	2.43	4.98	1.15	
	"	10^{-3}M	-0.43	-0.07	0.40	0.27	0.33	0.02	98.60
	"	10^{-4}M	-0.23	0.69	0.92	1.13	1.89	0.33	70.88
4	10^{-5}M	____	-0.17	0.54	1.71	1.89	4.18	0.87	
	"	10^{-3}M	-0.55	-0.22	1.43	1.18	1.79	0.15	83.33
	"	10^{-4}M	-0.05	-0.16	1.50	1.73	3.06	0.54	38.16

Effect of NaCN pretreatment on the uptake of IAA by Avena coleoptile segments.

TABLE VII

GROWTH SUBSTANCES		UPTAKE OF IAA (µg)							RATE	% I
IAA	NMSP	30 m	1 hr	2 hr	3 hr	4 hr	6 hr	8 hr		
5×10^{-6}M	_____	0	1.12	1.76	2.16	3.49	4.64	5.53	0.66	
"	5×10^{-6}M	0.24	0.64	1.11	2.08	2.87	3.46	4.60	0.56	14.4
"	10^{-5}M	0.52	0.79	1.58	1.86	2.68	3.69	4.56	0.54	18.4
"	2.5×10^{-5}M	0.19	0.34	0.67	1.47	2.23	2.85	3.75	0.49	24.6
"	5×10^{-5}M	0.47	0.96	1.39	1.33	2.29	2.92	3.42	0.37	44.0

Effect of NMSP on the uptake of IAA by Avena coleoptile segments.

TABLE VIII

GROWTH SUBSTANCES		UPTAKE OF IAA (µg)							RATE	% I
IAA	NMSP	30 m	1 hr	2 hr	3 hr	4 hr	6 hr	8 hr		
5×10^{-6}M	———	1.19	0.66	1.16	1.88	2.72	3.98	5.17	0.66	
"	5×10^{-6}M	0.35	0.82	1.63	2.05	2.95	3.76	5.08	0.59	10.6
"	10^{-5}M	0.35	0.35	0.92	1.51	1.96	2.85	3.58	0.46	30.3
"	2.5×10^{-5}M	0.56	0.36	1.19	1.42	1.81	2.76	3.48	0.43	35.2
"	5×10^{-5}M	0.77	0.37	1.13	1.37	1.53	2.28	2.86	0.33	49.8

Effect of NMSP on the uptake of IAA by Avena coleoptile segments.

TABLE IX

GROWTH SUBST.		UPTAKE OF IAA (µg)								RATE	% I
IAA	NMSP	15 m	30 m	1 hr	2 hr	3 hr	4 hr	6 hr	8 hr		
10^{-5}M	———	1.88	1.16	1.73	2.47	3.53	4.69	6.44	8.96	1.03	
"	5×10^{-6}M	1.13	0.84	1.67	3.12	3.92	4.63	6.28	8.79	0.96	7.17
"	10^{-5}M	0.38	0.89	1.82	2.88	3.72	4.69	6.15	8.58	0.93	9.69
"	2.5×10^{-5}M	0.98	0.81	1.89	3.12	3.76	4.14	5.54	7.49	0.75	27.71
"	5×10^{-5}M	1.25	2.20	2.43	3.06	3.46	4.00	5.25	6.95	0.63	38.85

Effect of NMSP on the uptake of IAA by Avena coleoptile segments.

TABLE X

GROWTH SUBSTANCES		UPTAKE OF IAA (µg)								RATE	% I
IAA	NMSP	15 m	30 m	1 hr	2 hr	3 hr	4 hr	6 hr	8 hr		
2.5×10^{-5}M	_____	0.73	3.15	4.79	4.79	10.04	10.58	13.14	18.18	1.91	
"	5×10^{-6}M	0	4.15	4.15	7.26	9.29	10.11	12.42	15.97	1.54	19.0
"	10^{-5}M	0.73	2.80	4.12	5.36	9.15	9.97	12.01	15.57	1.59	16.8
"	2.5×10^{-5}M	1.98	4.15	5.39	5.51	9.15	9.44	11.04	14.49	1.28	32.8
"	5×10^{-5}M	1.98	4.15	5.39	4.87	8.20	8.89	12.24	13.13	1.26	34.0

Effect of NMSP on the uptake of IAA by Avena coleoptile segments.

TABLE XI

GROWTH SUBSTANCES		UPTAKE OF IAA (µg)								RATE	% I
IAA	NMSP	15 m	30 m	1 hr	2 hr	3 hr	4 hr	6 hr	8 hr		
5×10^{-5}M	———	0.74	3.54	7.76	7.97	10.14	12.73	18.08	30.09	3.15	
"	5×10^{-6}M	-5.67	5.33	5.56	4.93	6.51	9.28	14.46	23.27	2.62	17.0
"	10^{-5}M	1.23	4.75	1.64	1.85	6.19	7.29	14.37	22.68	3.08	2.3
"	2.5×10^{-5}M	1.23	4.75	9.18	6.67	10.02	12.06	17.06	24.99	2.45	22.4
"	5×10^{-5}M	1.48	5.93	8.14	8.14	12.28	14.14	18.97	25.69	2.58	18.1

Effect of NMSP on the uptake of IAA by Avena coleoptile segments.

TABLE XII

GROWTH SUBSTANCES IAA	NMSP	UPTAKE OF IAA (µg) 30 m	1 hr	2 hr	4 hr	6 hr	RATE 1 - 6 hr	%I/S	RATE 0 - 6 hr	% I/S
10^{-5}M	_____	0.48	0.32	1.73	3.83	6.81	1.28		1.14	
"	10^{-5}M	0.76	0.82	1.89	4.15	6.76	1.19	-6.5	1.11	-2.6
"	2.5 x 10^{-5}M	0.00	0.45	1.89	4.20	6.09	1.12	-11.7	1.08	+4.7
"	5 x 10^{-5}M	-0.33	0.31	1.95	3.56	5.43	0.98	-22.6	0.98	-14.0

Effect of NMSP on the uptake of IAA by Avena coleoptile segments. In column 8, Rate of uptake is calculated from the uptake figures between 1 - 6 hours, and in column 10, from the figures between 0 - 6 hours.

TABLE XIII

GROWTH SUBSTANCES IAA	NMSP	UPTAKE OF IAA (µg) 30 m	1 hr	2 hr	4 hr	6 hr	RATE 1 - 6 hr	% I/S	RATE 0 - 6 hr	% I/S
2.5×10^{-5}M	———	0.81	1.92	3.20	8.06	11.17	1.93		1.91	
"	10^{-5}M	1.01	2.40	4.42	8.51	12.11	1.95	+1.1	2.03	+6.0
"	2.5×10^{-5}M	-0.22	2.16	2.96	8.46	11.58	2.01	+4.2	2.03	+6.0
"	5×10^{-5}M	0.62	2.58	2.86	5.62	10.44	1.60	-17.2	1.64	-14.4

Effect of NMSP on the uptake of IAA by Avena coleoptile segments. In column 8, Rate of uptake is calculated from the uptake figures between 1 - 6 hours, and in column 10, from the figures between 0 - 6 hours.

TABLE XIV

GROWTH SUBSTANCES IAA	NMSP	UPTAKE OF IAA (µg) 30 m	1 hr	2 hr	4 hr	6 hr	RATE 1 - 6 hr	% I/S	RATE 0 - 6 hr	% I/S
5×10^{-5}M	_____	3.99	4.19	6.06	16.13	24.70	4.27		3.99	
"	10^{-5}M	2.19	4.20	7.84	15.07	24.16	3.97	-7.2	3.94	-1.1
"	2.5×10^{-5}M	1.84	3.88	6.29	14.24	21.39	3.59	-16.0	3.55	-10.8
"	5×10^{-5}M	-0.18	1.81	2.41	9.58	16.96	3.17	-25.9	2.90	-27.4

Effect of NMSP on the uptake of IAA by Avena coleoptile segments. In column 8, Rate of uptake is calculated from the uptake figures between 1 - 6 hours, and in column 10, from the figures between 0 - 6 hours.

TABLE XV

GROWTH SUBSTANCES IAA	NMSP	UPTAKE OF IAA (µg) 30 m	1 hr	2 hr	4 hr	6 hr	RATE 1 - 6 hr	% I/S	RATE 0 - 6 hr	% I/S
5×10^{-5}M	_____	0.52	2.04	8.84	13.37	19.33	3.22		3.36	
"	2.5×10^{-5}M	-0.64	2.78	9.48	11.99	19.15	2.95	-8.4	3.24	-3.6
"	5×10^{-5}M	0.65	0.97	6.71	12.93	18.48	3.39	+5.0	3.27	-2.6
"	10^{-4}M	1.39	4.11	6.35	9.64	16.36	2.38	-26.3	2.57	-23.4

Effect of NMSP on the uptake of IAA by Avena coleoptile segments. In column 8, Rate of uptake is calculated from the uptake figures between 1 - 6 hours, and in column 10, from the figures between 0 - 6 hours.

TABLE XVI

NAA CONC.	UPTAKE (µg) 30 m	1 hr	4 hr	RATE
5×10^{-6} M	0.59	0.72	2.15	0.50
10^{-5} M	1.99	2.75	6.32	1.43
2.5×10^{-5} M	3.69	5.74	15.11	3.54
5×10^{-5} M	0.00	5.37	25.67	6.75

Uptake of NAA by Avena coleoptile segments. Rate of uptake was calculated from the uptake figures between 0 - 4 hours.

TABLE XVII

NAA	UPTAKE (µg)					RATE
	30 m	1 hr	2 hr	4 hr	6 hr	
5×10^{-6} M	0.49	0.54	1.84	3.39	4.69	0.77
10^{-5} M	1.70	1.81	4.31	8.03	10.57	1.64
2.5×10^{-5} M	2.41	3.38	10.01	19.13	26.45	4.21
5×10^{-5} M	4.08	8.26	19.27	37.25	51.43	8.16

Uptake of NAA by Avena coleoptile segments. Rate of uptake calculated from the uptake figures between 0 - 6 hours.

TABLE XVIII

NAA	UPTAKE (µg)				RATE
	30 m	1 hr	2 hr	4 hr	
10^{-5}M	1.49	1.73	3.07	5.15	1.20
2.5 x 10^{-5}M	4.28	6.06	10.02	14.59	3.42
5 x 10^{-5}M	9.41	8.61	17.71	26.97	6.26

Uptake of NAA by Avena coleoptile segments. Rate of uptake was calculated from the uptake figures between 0 - 4 hours.

TABLE XIX

NAA	RATE OF UPTAKE			MEAN	M.D.
	Expt. 1	Expt. 2	Expt. 3		
5×10^{-6}M	0.50	0.77		0.64	0.14
10^{-5}M	1.43	1.64	1.20	1.42	0.15
2.5×10^{-5}M	3.54	4.21	3.42	3.72	0.33
5×10^{-5}M	6.75	8.16	6.26	7.05	0.74

Comparison of the rates of uptake of NAA obtained from the three experiments represented in Tables XVI - XVIII.

TABLE XXI

NAA	2,4-D	NMSP	UPTAKE OF NAA (µg)					RATE
			30 m	1 hr	2 hr	4 hr	6 hr	
10^{-5}M	———	———	1.17	1.60	2.72	4.82	6.49	1.04
"	10^{-5}M	———	1.17	1.94	2.89	5.22	6.62	1.07
"	2.5 x 10^{-5}M	———	0.54	1.19	2.54	4.52	6.42	1.07
"	———	10^{-5}M	0.00	1.27	2.42	4.49	6.39	1.10

Effect of 2,4-D and NMSP on the uptake of NAA by Avena coleoptile segments.
Rate of uptake ___ 0 - 6 hours.

TABLE XXII

NAA	2,4-D	NMSP	UPTAKE OF NAA (µg)					RATE
			30 m	1 hr	2 hr	4 hr	6 hr	
10^{-5}M	_____	_____	0.85	1.31	2.72	4.71	6.58	1.08
"	5 x 10^{-5}M	_____	0.73	1.27	2.71	4.81	6.32	1.06
"	_____	2.5 x 10^{-5}M	0.52	1.42	3.37	4.67	6.22	1.29
"	_____	5 x 10^{-5}M	0.80	1.49	3.12	3.86	5.31	0.84

Effect of 2,4-D and NMSP on the uptake of NAA by Avena coleoptile segments. Rate of uptake ___ 0 - 6 hours.

TABLE XXIII

NAA	2,4-D	NMSP	UPTAKE OF NAA (µg)					RATE
			30 m	1 hr	2 hr	4 hr	6 hr	
2.5×10^{-5}M	_____	_____	1.54	2.42	6.88	11.28	15.39	2.61
"	10^{-5}M	_____	1.54	1.54	5.07	11.12	15.76	2.70
"	2.5×10^{-5}M	_____	0.88	1.13	4.19	10.25	13.54	2.41
"	_____	2.5×10^{-5}M	0.75	1.99	3.99	10.38	13.58	2.39

Effect of 2,4-D and NMSP on the uptake of NAA by Avena coleoptile segments.
Rate of uptake _____ 0 - 6 hours.

TABLE XXIV

IAA	Expt. NO.	UPTAKE OF IAA (µg) 30 m	1 hr	2 hr	4 hr	5 hr	6 hr	7 hr	RATE
5×10^{-6}M	1	0.47	0.67		1.31		1.88		0.25
10^{-5}M	1	0.45	1.13	1.07	2.34		3.17		
	2		0.58	1.16	2.31			5.01	
	3	1.79	2.04	3.44	4.15		5.19		
	4	0.76	1.11	1.62	3.05		4.69		
	5	0.30	0.82	1.34	2.71		3.98		
	6			1.78		3.58	4.30		
	MEAN	0.82	1.14	1.74	2.91	3.58	4.27		0.62
	M.D.	0.48	0.36	0.58	0.55		0.56		

Uptake of IAA by mesocotyl segments of Zea mays L.

TABLE XXV

IAA	Expt. No.	UPTAKE OF IAA (µg)							RATE
		30 m	1 hr	2 hr	3 hr	4 hr	6 hr	24 hr	
2.5×10^{-5} M	1	2.62	4.12	7.38		10.00	13.27	25.05	
	2			8.66	11.54	13.12	15.09		
	3	5.36	5.93	9.12		11.40			
	4	8.75	9.27	13.25		15.89	19.30		
	5	2.82	4.03	4.92		10.20	10.99		
	MEAN	4.89	5.83	8.66		12.12	14.66		1.79
	M.D.	2.17	1.76	2.01		1.91	2.53		

Uptake of IAA by mesocotyl segments of Zea mays L.

TABLE XXVI

IAA	Expt. No.	UPTAKE OF IAA (µg) 30 m	1 hr	2 hr	4 hr	6 hr	RATE
5×10^{-5}M	1	6.47	7.23	12.86	18.53	22.26	
	2	6.08	11.62	15.88	21.89	24.80	
	MEAN	6.28	9.42	14.37	20.21	23.53	3.08
	M.D.	0.19	2.19	1.51	1.68	1.27	
10^{-4}M	1	17.47	11.64	22.70	28.29	33.56	
	2	9.76	18.26	25.22	35.59	36.90	
	MEAN	13.62	14.95	23.96	31.94	35.23	4.12
	M.D.	3.86	3.31	1.26	3.65	1.67	

Uptake of IAA by mesocotyl segments of Zea mays L.

TABLE XXVII

SAMPLE	UPTAKE OF IAA (in CPM)					
	1 hr	2 hr	4 hr	5 hr	6 hr	8 hr
A	627	844	1537	1772	1893	2606
B	Pretreated with 'cold' IAA (5×10^{-5} M)			478	959	1437
C	Pretreated with IAA-C^{14}			1170	1373	933

Uptake of IAA by mesocotyl segments of Zea mays L.

TABLE XXVIII

TIME	FLUORESCENCE			UPTAKE OF IAA (μg)		
	A	B	C	A	B	C
In (b)	44.0	44.5	45.3	0	0	0
30 m	42.0	41.9	43.0	0.76	0.97	0.85
1 hr	41.0	40.2	41.5	1.11	1.57	1.37
2 hr	39.5	38.0	39.0	1.62	2.31	2.18
4 hr	35.0	33.0	35.0	3.05	3.88	3.42
6 hr	29.5	28.2	30.1	4.69	5.29	4.84

Effect of 2,4-D and NMSP on the uptake of IAA by Zea mays mesocotyl segments.

A = IAA (10^{-5}M);

B = IAA (10^{-5}M) + NMSP (2.5×10^{-5}M);

C = IAA (10^{-5}M) + 2,4-D (5×10^{-5}M).

TABLE XXIX

TIME	FLUORESCENCE			UPTAKE OF IAA (µg)		
	A	B	C	A	B	C
In (b)	43.7	41.8	41.8	----	----	----
30 m	39.0	39.0	38.5	1.79	1.11	1.31
1 hr	38.3	38.2	36.8	2.04	1.42	1.95
2 hr	34.2	34.5	32.6	3.44	2.73	3.45
4 hr	32.0	31.0	30.8	4.15	3.90	4.06
6 hr	28.5	29.3	28.2	5.19	4.43	4.86

Effect of NMSP on the uptake of IAA by Zea mesocotyl segments.
A = IAA (10^{-5}M); B = IAA (10^{-5}M) + NMSP (10^{-5}M);
C = IAA (10^{-5}M) + NMSP (5×10^{-5}M).

TABLE XXX

TIME	FLUORESCENCE		UPTAKE OF IAA (µg)	
	A	B	A	B
In (b)	34.0	34.0	0	0
30 m	28.0	30.5	8.75	4.05
1 hr	27.5	28.5	9.27	6.24
2 hr	23.4	25.0	13.25	9.84
4 hr	20.5	21.5	15.90	13.22
6 hr	16.5	19.0	19.30	15.47

Effect of NMSP on the uptake of IAA by *Zea* mesocotyl segments. A = IAA (2.5×10^{-5}M); B = IAA (2.5×10^{-5}M) + NMSP (2.5×10^{-5}M).

TABLE XXXI

IAA	NPA	FLOURESCENCE INTENSITY						RATE	% S
		In (b)	30 m	1 hr	2 hr	4 hr	6 hr		
10^{-5}M	_____	46.2	45.5	45.2	43.5	40.0	39.5	1.18	
"	10^{-5}M	47.5	45.5	46.5	42.0	36.6	35.0	2.27	91.62
"	2.5 x 10^{-5}M	47.5	47.1	45.5	43.0	38.0	36.0	1.94	64.21
"	5 x 10^{-5}M	47.0	44.9	42.5	39.7	36.0	35.5	1.39	18.27
"	10^{-4}M	43.0	42.0	40.0	37.5	33.5	31.5	1.70	44.16

Effect of NPA on the uptake of IAA by Zea mesocotyl segments in terms of decreasing fluorescence intensity.

TABLE XXXII

IAA	NPA	FLUORESCENCE INTENSITY					RATE	% I/S
		In (b)	30 m	1 hr	2 hr	5 hr		
2.5×10^{-5}M	_____	28.0	28.6	27.9	27.6	23.2	1.24	
"	10^{-5}M	28.5	28.8	28.0	26.0	21.0	1.73	+39.37
"	2.5×10^{-5}M	28.1	28.2	26.5	25.0	21.4	1.26	+ 1.21
"	5×10^{-5}M	27.0	26.0	25.6	24.5	21.2	1.10	-11.35
"	10^{-4}M	24.6	24.7	23.8	22.5	19.0	1.19	- 4.11

Effect of NPA on the uptake of IAA by Zea mesocotyl segments in terms of decreasing fluorescence intensity.

TABLE XXXIII

IAA	NPA	FLUORESCENCE INTENSITY						RATE	% S
		In (b)	30 m	1 hr	2 hr	4 hr	6 hr		
2.5×10^{-5}M	_____	43.0	41.5	39.0	37.2	35.3	33.0	1.16	
"	10^{-5}M	41.5	40.0	38.0	36.0	32.0	29.0	1.82	57.14
"	2.5×10^{-5}M	40.0	39.3	36.5	33.5	32.0	___	___	____
"	5×10^{-5}M	39.0	37.0	35.5	32.5	29.0	24.5	2.13	84.16
"	10^{-4}M	36.0	35.5	34.0	31.9	28.1	26.0	1.61	39.74

Effect of NPA on the uptake of IAA by Zea mesocotyl segments in terms of decreasing fluorescence intensity.

TABLE XXXIV

IAA	NPA	* FLUORESCENCE INTENSITY						RATE	% S
		IN (b)	30 m	1 hr	2 hr	4 hr	6 hr		
5×10^{-5}M	_____	21.2	20.3	20.3	19.7	18.2	16.4	0.87	
"	10^{-5}M	20.7	20.3	20.4	19.1	17.7	15.3	1.09	+24.4
"	2.5×10^{-5}M	20.9	20.1	19.3	18.5	16.8	14.4	1.07	+23.0
"	5×10^{-5}M	20.4	19.2	18.5	17.4	15.9	14.6	0.83	-5.5
"	10^{-4}M	18.0	16.7	16.8	16.3	14.6	13.2	0.81	-7.6

Effect of NPA on the uptake of IAA by Zea mesocotyl segments in terms of decreasing fluorescence intensity.

TABLE XXXV

IAA	NPA	FLUORESCENCE INTENSITY						RATE	% I/S
		In (b)	30 m	1 hr	2 hr	4 hr	6 hr		
10^{-4}M	_____	42.0	39.5	37.1	34.1	33.3	29.5	1.36	
"	10^{-5}M	41.0	39.0	37.8	35.2	32.0	28.9	1.73	+27.88
"	5 x 10^{-5}M	38.0	37.0	35.0	33.9	30.7	28.0	1.43	+ 5.53
"	10^{-4}M	35.5	33.3	32.2	31.0	28.0	26.6	1.27	- 6.42

Effect of NPA on the uptake of IAA by Zea mesocotyl segments in terms of decreasing fluorescence intensity.

TABLE XXXVI

IAA	NPA	IAA UPTAKE (CPM) 1 hr	2 hr	4 hr	6 hr	8 hr	RATE	% S
10^{-5}M	_____	128	181	281	406	559	60.70	
"	10^{-5}M	163	254	481	547	674	72.26	19.04
"	5 x 10^{-5}M	190	285	457	627	782	84.57	39.33
"	10^{-4}M	185	256	445	586	769	83.32	37.27

Effect of NPA on the uptake of IAA - C^{14} by Zea mesocotyl segments.

TABLE XXXVII

IAA	NPA	IAA UPTAKE (CPM)					RATE 1 - 8 hr	% S/I	RATE 1-24 hr	% S
		1 hr	2 hr	4 hr	8 hr	24 hr				
10^{-4}M	_____	1158	1846	2992	4533	8455	473		302	
"	10^{-5}M	1170	1746	2954	4797	9816	516	+ 9.17	366	21.07
"	5 x 10^{-5}M	1209	1799	2971	4252	10445	429	- 9.30	393	29.84
"	10^{-4}M	1046	1555	2956	5239	9884	605	+27.97	375	23.99

Effect of NPA on the uptake of IAA-C^{14} by Zea mesocotyl segments.

TABLE XXXVIII

IAA	TIBA	UPTAKE OF IAA (µg)					RATE	% I/S	% I/S (Total Uptake)
		30 m	1 hr	2 hr	4 hr	6 hr			
10^{-5}M	———	0.30	0.82	1.34	2.71	3.98	0.66		
"	10^{-5}M	-0.18	-0.05	0.89	3.07	4.04	0.85	+29.19	———
"	5 x 10^{-5}M	-0.60	-0.83	-0.50	0.54	0.88	0.32	-50.98	-77.9
"	10^{-4}M	-0.32	-1.48	-0.44	-0.17	0.47	0.31	-52.35	-88.2

Effect of TIBA on IAA uptake by Zea mesocotyl segments. Rate:- 0 __ 6 hours.

TABLE XXXIX

IAA	TIBA	UPTAKE OF IAA (µg)					RATE	% I/S
		30 m	1 hr	2 hr	4 hr	6 hr		
2.5×10^{-5}M	———	2.82	4.03	4.92	10.19	10.99	1.54	
"	10^{-5}M	3.17	2.65	6.48	9.87	11.19	1.64	+ 6.45
"	2.5×10^{-5}M	0.55	3.15	5.12	7.93	8.91	1.15	-25.20
"	5×10^{-5}M	3.86	5.32	5.67	6.22	7.35	0.39	-74.22
"	10^{-4}M	1.99	3.76	3.09	3.72	5.42	0.37	-76.17

Effect of TIBA on the uptake of IAA by Zea mesocotyl segments.

TABLE XL

IAA	TIBA	UPTAKE OF IAA (µg)					RATE	% I
		30 m	1 hr	2 hr	4 hr	6 hr		
5×10^{-5}M	———	6.47	7.23	12.86	18.53	22.26	2.90	
"	10^{-5}M	0.87	9.10	9.10	14.99	20.22	2.37	18.12
"	5×10^{-5}M	2.87	7.94	9.74	9.85	15.07	1.27	56.11
"	10^{-4}M	1.54	6.60	7.21	8.34	8.55	0.40	86.23

Effect of TIBA on the uptake of IAA by Zea mesocotyl segments.

TABLE XLI

IAA	TIBA	UPTAKE OF IAA (µg)					RATE	% I
		30 m	1 hr	2 hr	4 hr	6 hr		
10^{-4}M	———	17.47	11.64	22.70	28.29	33.56	3.99	
"	10^{-5}M	8.26	6.18	18.42	23.64	27.15	3.76	5.93
"	5 x 10^{-5}M	4.75	5.59	13.50	14.98	19.19	2.34	41.37
"	10^{-4}M	-6.22	-0.81	7.49	10.85	10.85	2.06	48.42

Effect of TIBA on the uptake of IAA by Zea mesocotyl segments.

TABLE XLII

IAA	TIBA	UPTAKE OF IAA (µg)					RATE	% I
		30 m	1 hr	2 hr	4 hr	6 hr		
5×10^{-5}M	———	6.08	11.62	15.88	21.89	24.80	2.62	
"	2.5×10^{-5}M	3.41	8.84	12.44	16.78	18.63	1.92	26.55
10^{-4}M	———	9.76	18.26	25.22	35.60	36.90	3.77	
"	2.5×10^{-5}M	7.32	19.69	23.72	33.40	33.87	3.00	20.36

Effect of TIBA on the uptake of IAA by Zea mesocotyl segments.

TABLE XLIII

IAA	TIBA	IAA UPTAKE (CPM)					RATE	% I/S
		1 hr	2 hr	4 hr	6 hr	7½ hr		
10^{-5}M	———	96	147	266	317	468	53.8	
"	10^{-5}M	108	178	288	450	527	65.4	+21.6
"	2.5 x 10^{-5}M	111	180	271	351	466	51.4	- 4.4
"	5 x 10^{-5}M	80	150	223	296	343	39.2	-27.2
"	10^{-4}M	73	109	161	214	271	29.4	-45.5

Effect of TIBA on the uptake of IAA - C^{14} by Zea mesocotyl segments.

TABLE XLIV

IAA	TIBA	IAA UPTAKE (CPM) 1 hr	2 hr	4 hr	6 hr	RATE	% I/S
10^{-5}M	_____	107	159	303	363	53.3	
"	10^{-5}M	116	206	307	406	56.1	+ 5.4
"	2.5 x 10^{-5}M	105	182	259	361	49.0	- 8.0
"	5 x 10^{-5}M	112	135	206	295	36.9	-30.7
"	10^{-4}M	79	115	163	232	29.8	-44.2

Effect of TIBA on the uptake of IAA - C^{14} by Zea mesocotyl segments.

TABLE XLV

IAA	TIBA	IAA UPTAKE (CPM) 1 hr	2 hr	4 hr	6 hr	RATE	% I
2.5×10^{-5}M	_____	355	492	935	1217	178.6	
"	10^{-5}M	335	523	914	1029	142.9	19.9
"	2.5×10^{-5}M	299	417	737	717	90.0	49.6
"	5×10^{-5}M	257	378	497	643	73.0	59.1
"	10^{-4}M	223	315	461	585	72.0	59.7

Effect of TIBA on the uptake of IAA - C^{14} by Zea mesocotyl segments.

TABLE XLVI

IAA	TIBA	IAA UPTAKE (CPM) 1 hr	2 hr	4 hr	6 hr	8 hr	RATE	% I
5×10^{-5}M	_____	519	950	1455	1902	2396	258.8	
"	10^{-5}M	626	891	1391	1698	2048	201.1	23.3
"	2.5×10^{-5}M	365	660	1038	1505	____	221.8	14.3
"	5×10^{-5}M	391	556	837	1069	1153	111.7	56.8
"	10^{-4}M	309	424	626	799	____	97.7	62.2

Effect of TIBA on the uptake of IAA - C^{14} by Zea mesocotyl segments.

TABLE XLVII

IAA	TIBA	IAA UPTAKE (CPM)				RATE	% I
		1 hr	2 hr	4 hr	6 hr		
10^{-4}M	_____	1254	2787	3869	4153	543.5	
"	10^{-5}M	1168	1935	3306	3792	532.9	1.9
"	2.5 x 10^{-5}M	1008	1526	2349	3136	421.0	22.5
"	5 x 10^{-5}M	764	1239	1809	2562	348.0	35.9
"	10^{-4}M	668	1055	1601	1667	200.9	63.0

Effect of TIBA on the uptake of IAA - C^{14} by Zea mesocotyl segments.

TABLE XLVIII

SAMPLE	IAA UPTAKE (CPM)				% Inhib. of Total Uptake
	1 hr	2 hr	4 hr	6 hr	
A	674	1000	1579	2269	
B	250	377	530	818	63.95
C	448	717	1128	1701	25.03
D	230	321	508	806	64.48

Effect of DNP on the TIBA - induced inhibition of IAA - C^{14} uptake by *Zea* mesocotyl segments. A = IAA (5×10^{-5}M); C = IAA (5×10^{-5}M); B = IAA (5×10^{-5}M) + TIBA (5×10^{-5}M); D = IAA (5×10^{-5}M) + TIBA (5×10^{-5}M).

'C' and 'D' were pretreated for one hour with DNP (10^{-4}M).

TABLE XLIX

IAA	TIBA	DNP	IAA UPTAKE (CPM) 1 hr	2 hr	4 hr	6 hr	% Inhib of Uptake
5×10^{-5}M	———	———	646	1106	1777	2422	
"	5×10^{-5}M	———	235	335	529	820	66.14
"	———	10^{-4}M	506	708	967	1058	56.32
"	5×10^{-5}M	10^{-4}M	189	275	389	498	79.44

Effect of DNP on the TIBA - induced inhibition of IAA - C^{14} uptake by Zea mesocotyl segments.

TABLE L

IAA	TIBA	BAL	IAA UPTAKE (CPM)				RATE
			1 hr	2 hr	4 hr	6 hr	
5×10^{-5}M	____	___	650	870	1500	1973	271
"	10^{-4}M	___	215	351	578	671	92
"	____	10^{-4}M	694	958	1718	1986	271
"	10^{-4}M	10^{-4}M	222	340	579	627	84

Effect of BAL on the TIBA - induced inhibition of IAA - C^{14} uptake by Zea mesocotyl segments.

TABLE LI

IAA	TIBA	BAL	IAA UPTAKE (CPM)				RATE
			1 hr	2 hr	4 hr	6 hr	
5×10^{-5}M	_____	___	630	1052	1652	2140	297
"	5×10^{-5}M	___	422	601	934	1405	194
"	_____	10^{-4}M	624	1093	1622	2193	304
"	5×10^{-5}M	10^{-4}M	526	796	1014	1343	154

Effect of BAL on the TIBA - induced inhibition of IAA - C^{14} uptake by Zea mesocotyl segments.

TABLE LII

IAA	TIBA	BAL	IAA UPTAKE (CPM)				RATE
			1 hr	2 hr	4 hr	6 hr	
5×10^{-5}M	_____	____	577	1148	1611	2277	321
"	2.5×10^{-5}M	____	477	826	1257	1540	208
"	_____	10^{-4}M	662	1101	1451	2376	322
"	2.5×10^{-5}M	10^{-4}M	479	779	1243	1574	217

Effect of BAL on the TIBA - induced inhibition of IAA - C^{14} uptake by Zea mesocotyl segments.

TABLE LIII

IAA	TIBA	CYST.	IAA UPTAKE (CPM)				RATE
			1 hr	2 hr	4 hr	6 hr	
5×10^{-5}M	_____	_____	701	1244	1604	2332	304
"	5×10^{-5}M	_____	449	799	1112	1564	212
"	_____	10^{-4}M	653	1220	1573	2420	328
"	5×10^{-5}M	10^{-4}M	451	738	980	1397	179

Effect of cysteine on the TIBA - induced inhibition of IAA - C^{14} uptake by Zea mesocotyl segments.

TABLE LIV

IAA	IOXYNIL	IAA UPTAKE (CPM)				RATE	% I
		1 hr	2 hr	4 hr	6 hr		
10^{-5}M	_____	110	170	284	324	43.66	
"	10^{-5}M	106	147	198	283	34.17	21.74
"	5 x 10^{-5}M	79	108	145	167	17.29	60.40
"	10^{-4}M	65	82	95	130	12.20	72.05

Effect of Ioxynil on the uptake of IAA - C^{14} by Zea mesocotyl segments.

TABLE LV

IAA	IOXYNIL	IAA UPTAKE (CPM)				RATE	% I
		1 hr	2 hr	4 hr	6 hr		
5×10^{-5}M	———	536	890	1349	2019	287.80	
"	10^{-5}M	440	677	1042	1346	179.46	37.64
"	5×10^{-5}M	373	530	648	834	86.64	69.89
"	10^{-4}M	290	395	582	631	69.49	75.85

Effect of Ioxynil on the uptake of IAA - C^{14} by Zea mesocotyl segments.

TABLE LVI

IAA	IOXYNIL	IAA UPTAKE (CPM)				RATE	% I
		1 hr	2 hr	4 hr	6 hr		
10^{-4}M	_____	905	1638	2765	3514	518.85	
"	10^{-5}M	779	1197	1576	2363	300.41	42.10
"	5 x 10^{-5}M	625	862	1249	1538	181.83	64.96
"	10^{-4}M	521	786	1059	1229	136.88	73.62

Effect of Ioxynil on the uptake of IAA - C^{14} by Zea mesocotyl segments.

TABLE LVII

IAA	BROM--OXYNIL	IAA UPTAKE (CPM) 1 hr	2 hr	4 hr	6 hr	RATE	% I
10^{-5}M	_____	85	146	246	301	43.25	
"	10^{-5}M	89	133	220	278	38.17	11.76
"	5 x 10^{-5}M	85	117	173	189	21.15	51.09
"	10^{-4}M	76	102	140	159	16.54	61.76

Effect of Bromoxynil on the uptake of IAA - C^{14} by Zea mesocotyl segments.

TABLE LVIII

MEDIA	FLUORESCENCE INTENSITY						
	In (a)	In (b)	30 m	1 hr	2 hr	4 hr	6 hr
Buffer	0.76	0.98	4.65	9.15	11.85	15.60	23.50
Distilled water	0.66	1.41	3.60	4.85	5.30	5.10	4.10
IAA (10^{-5}M) in Buffer	38.10	40.50	38.00	37.00	37.00	37.00	37.00
IAA (10^{-5}M) in Dist. Water	46.50	47.50	46.00	45.50	42.50	32.50	18.00

Uptake of IAA by mung bean hypocotyl segments in terms of decreasing fluorescence intensity. 50 segments per 10 ml solution.

TABLE LIX

MEDIA	FLUORESCENCE INTENSITY						
	In (a)	In (b)	30 m	1 hr	2 hr	4 hr	6 hr
Buffer	0.60	1.29	5.80	9.81	17.70	28.00	33.00
Distilled Water	0.84	0.96	3.35	4.45	6.30	6.85	6.40
IAA (10^{-5}M) in Buffer	47.00	49.50	47.00	46.10	49.00	52.50	56.00
IAA (10^{-5}M) in Dist. Water	50.00	50.00	50.00	47.80	47.00	39.00	27.50

Uptake of IAA by mung bean hypocotyl segments in terms of decreasing fluorescence intensity. 50 segments per 10 ml solution.

TABLE LX

IAA	IAA UPTAKE (CPM)				RATE
	1 hr	2 hr	4 hr	6 hr	
10^{-5}M	256	337	511	529	63.45
2.5×10^{-5}M	803	1036	1062	1550	147.72
5×10^{-5}M	1749	1920	2000	2959	249.21
10^{-4}M	3602	3666	4847	4876	328.85

Uptake of IAA - C^{14} by Phaseolus hypocotyl segments.

TABLE LXI

IAA	TIBA	IAA UPTAKE (CPM) 1 hr	2 hr	4 hr	6 hr	RATE	% I
10^{-5}M	_____	262	340	531	699	89.49	
"	10^{-5}M	315	372	479	675	70.64	21.06
"	2.5 x 10^{-5}M	276	305	281	306	3.39	96.21
"	5 x 10^{-5}M	266	295	236	203	-15.73	117.58

Effect of TIBA on the uptake of IAA - C^{14} by Phaseolus hypocotyl segments.